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THE RELATIONS OF BURSARIA TO FOOD

II. DIGESTION AND RESORPTION IN THE FOOD VACUOLE, AND FURTHER ANALYSIS OF THE PROCESS OF EXTRUSION

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EIGHT FIGURES AND TWO PLATES

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INTRODUCTION

To obtain exact information on the processes of metabolism in single-celled organisms, and particularly the Protozoa, experimental procedure must be based upon simple, well defined and reproducible conditions, so that the work may be repeated and verified. Attempts to fulfill this requirement are subject to the

danger that the conditions may become so artificial as not to warrant conclusions concerning what occurs under usual ('normal') conditions of the organism. Nevertheless, a certain degree of simplicity may often be obtained in the conditions of experimentation and yet coincide with the usual conditions of a varied environment to a sufficient degree so that the results give a definite answer to questions which are directed toward finding out what the principles are which underlie the processes. The following experiments have been carried out with an attempt to fulfill the above requirements.

In a previous paper (Lund '14) some of the most important external relations of *Bursaria* to food have been presented, including a demonstration of a selective extrusion among the food vacuoles.

The following paper deals with the processes which take place in the food vacuole, and with the conditions under which it exists as an active system in this unicellular animal.

Proteins, fats, and carbohydrates in the form of starches are present in the material eaten by *Bursaria*, as may easily be detected by direct observation or by the aid of microchemical tests. Protein in the form of other living ciliates, flagellates, etc., is taken in and digested. Many of these *Protozoa* which serve as food contain fat globules and starch or amyllum grains, and hence all three food substances may at times be found present in the cytoplasm of *Bursaria* from flourishing cultures.

Since there is disagreement between results of different investigators, working with the same *Infusoria*; but more particularly because there are indications that similar forms among these animals differ in their food metabolism, the statements here made refer only to *Bursaria* and may or may not be valid for others, except where specifically stated.

MATERIAL AND METHODS

Simple types of the three classes of food were found in lipid-free egg yolk (vitellin), pure olein, and starch grains of various kinds. Potato starch was the most serviceable because grains of a very uniform size can be obtained by repeated decantation of a suspension, and hence results may be expressed quantitatively in terms of unit volume. Fresh hard boiled yolk—a combination, chiefly, of lipoids and protein—was also used. Olive oil of the best grade obtainable gave the same results as prepared olein.

From the yolk of the hen's egg was prepared a protein (vitellin) that was perfectly lipid-free, so far as could be determined; this was done as follows: An egg was boiled to hardness (15-20 minutes), and the granular portion of the yolk was kneaded gently to a moist, fine, floury pulp. This was placed loosely in a Soxhlet apparatus and extracted with an alcohol-ether mixture, for eight to ten hours; in this way the fat and lecithin were removed. The vitellin was then washed several times with fresh alcohol-ether mixture, removed from the Soxhlet and while still moist, gently kneaded to a fine white powder. This was left to dry at 25°C. for twenty-four hours. After drying was completed the residue was gently rubbed between sheets of filter-paper. When the steps mentioned above were carefully carried out, the grains of vitellin were found to be separated. This white dry powder was kept in a dry bottle in a cool place, and used as a food stock.

When the animals were to be fed, a suspension of the powder was made in tap or distilled water and rubbed up; then left to settle, and the supernatant liquid with the smaller particles was poured off. This process of separating the smaller grains from the larger ones was repeated until a perfectly clear suspension of the protein replicas of the uniform hard-boiled grains was obtained.

It is important to note that some eggs yield much more uniform yolk grains after boiling, than others. Only those eggs were used in this work which were satisfactory in this respect.

It seems highly probable from the results, that all traces of lecithin and fat had been removed by this method. Cold ether or cold alcohol extraction alone is not sufficient to remove all the lipid content from hard boiled yolk. This will appear from results (p. 29) of experiments in which such vitellin grains were used.

By this method of preparation, lipid-free vitellin may be obtained in very uniform grains, and this permits one to fulfill a condition most important for precise experimentation. One can feed definite unit quantities to a single cell, and this makes it possible to attack a number of questions regarding metabolism that would otherwise be inaccessible, and to express the results in quantitative terms.

Fresh hard-boiled yolk served as a protein-lipoid diet, which could be fed in the same way, for the vitellin grains are simply the protein matrix of the fresh hard boiled yolk grains in which the lipoids and any other alcohol-ether soluble substances are imbedded.

There is, of course, no means of knowing whether the vitellin of the unextracted yolk grain does not undergo change during ether-alcohol extraction; but the fact (demonstrated by the experiments) that in the food vacuole digestion of the protein in these two forms occurs in the same way and with about equal readiness, shows that the process of fat extraction does not alter the chemical or physical nature in such a way as to interfere with the digestion and resorption of the vitellin.

In all the experiments where necessary, the animals were placed in 500 cc. of tap water twenty-four hours previous to feeding. The result of this was that the food and debris contained in the cell had been discharged during the twenty-four hours of starvation, and hence a cell with perfectly clear cytoplasm was obtained for the experiments. By this means a particularly desirable uniformity in physiological condition of the organisms was obtained. The material was from the same wild stock cultures as that used in the experiments reported in my earlier paper.

In most of the experiments the organisms were fed singly, and large numbers were used, so that any individual variations play a minor part, if any, in the final results.

Where temperature regulation was necessary the experiments were carried on in an oven kept constant to within 1.5°C .

TO WHAT EXTENT CAN YOLK BE CONSIDERED A FOOD FOR BURSARIA

As yet it has not become possible to propagate pure races of *Bursaria* from single individuals, or in culture, by feeding it an artificial diet of vitellin or fresh yolk; so that it must be remembered in conjunction with the facts presented at this time, that the full requirements for the maintenance of life and reproduction have not been fully satisfied, and to this extent the ideal conditions have not been met.

But to prove that vitellin and fresh yolk are drawn upon for the energy requirements and growth of the cell Experiment I is given:

Experiment 1. Three sets of 74 individuals each were starved in tap water for 24 hours. Set A was fed with fresh hard boiled yolk in suspension prepared as described above. Set B was fed vitellin; while Set C was not fed. Each individual in the three sets was left to eat as much as it would in 20 minutes. They were then picked out and washed once in tap water which had been boiled, from this they were removed to watch glasses each containing 2 cc. of boiled tap water. The water was boiled in order to kill any bacteria, or other organisms sometimes present in small numbers in the tap water. Two individuals were placed in each watch glass and these were set in moist chambers which were kept side by side. There was therefore no difference in the temperature at which the three sets remained during the experiment, although a daily fluctuation in temperature of 4 or 5°C . occurred. Such variation, however, does not affect the results of the experiment for the particular end in view. It was found that after feeding twenty minutes nearly all of those in the fresh yolk suspension (A) had eaten large quantities, while those in the vitellin suspension (B) had on the average not eaten so large a number of grains as those in the fresh yolk suspension, although in both Set A and Set B, each individual had eaten more than one grain. All the individuals in the three sets were treated identically except in feeding. All were normal and active at the beginning of the experiment. At the end of every 24 hours detailed records were taken for each individual, as to whether it had a normal form or had undergone dedifferentiation—

which is under many conditions a typical reaction—and if the latter were the case, to what extent it had proceeded. Death was taken to have occurred when the cell had ceased to move and began to disintegrate. In this way a record of the effects of the food on the maintenance of form, degree of activity and length of life, was obtained, so that from these the means can be taken and compared.

To save space, instead of giving the detailed results in the form of tables they are given by curves and averages. Curves *A*, *B* and *C* of figure 1 represent for the three sets respectively, the longevity or death rate. Points on the abscissa indicate the time in hours, while points on the ordinate represent the number of individuals which were still alive at the time the record was taken.

The curves *A* and *B* of Sets A and B respectively, show clearly (1) that the yolk and vitellin had a definite effect in prolonging the life of the cell. The relation of curves *A* and *B* to each other will become clearer when we have considered later experiments (p. 28) which show, other things being equal, that we should expect longer life from individuals fed both lipoids and protein. By comparing the average length of life in the three sets we find that Set A lived 4.98, Set B, 5.39 and Set C 3.20 days. Sets A and B, therefore, lived on the average about two days longer than the unfed Set C. (2) The vitellin grains underwent total digestion and resorption in most cases, while the fresh yolk grains were generally only partially digested, this was especially the case where more than 2 or 3 grains were eaten. (3) The animals which had been fed were on the average more vigorous than those of Set C. (4) The animals of Set A grew to be larger in most cases than those of either Set B, or Set C. Many of Set A became twice as large as those of Set C, while those fed vitellin were on the average larger than any of Set C. The maximum size was reached about thirty-six hours after feeding.

There can therefore be no question but that both the fresh yolk and the vitellin entered into the chain of metabolic processes and were in part at least, drawn upon by the cell for its energy requirement. But very few of the organisms divided. Whether yolk or vitellin is a sufficient diet for cell division as

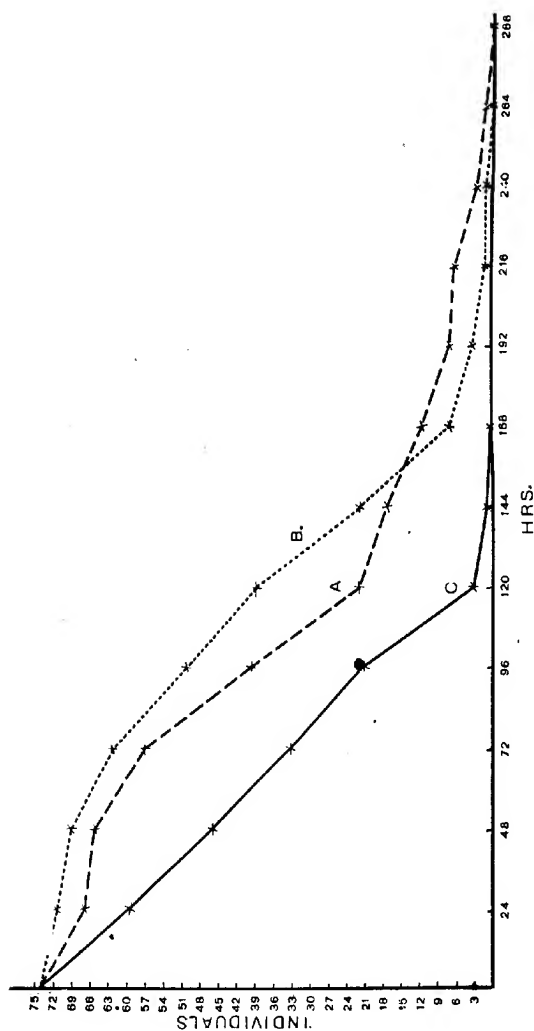


Fig. 1. Curves showing the length of life in three sets of 74 individuals each. Set A (Curve A) was fed fresh yolk; Set B (Curve B) was fed vitellin, while Set C (Curve C) was not fed.

well as for growth can not be answered at present. But for the questions dealt with in the present paper the important fact to establish is that the yolk or vitellin can be drawn upon for the energy requirements and growth of *Bursaria*.

PROTEIN DIGESTION

1. The food vacuole; formation and physical changes

The passage of the grain into the body is brought about partially by ciliary action at the base of the buccal pouch; but large grains or masses of food are pushed through the base of the gullet and into the endoplosm, by what seem to be contractions of the wall of the base of the gullet, and also apparently by activity of the endoplasm about the gullet behind the food. During this process of swallowing, a liquid comes to be included about the food so that when the vacuole separates from the base of the gullet liquid surrounds the yolk grain.

Where does the liquid enclosed with the grain come from? Part of it is derived from the external medium, as is readily determined by direct observation of the process of swallowing. But the liquid in the vacuole, when the latter is separated from the base of the gullet, is likewise partially made up of an acid secretion from the cytoplasm of the lower portion of the gullet, as will be demonstrated below. When the vacuole has formed it is usually carried toward the middle of the cell and may remain practically stationary, especially if it is large. If movement takes place, which is nearly always the case when the vacuole is small, then the vacuole may traverse any part of the cytoplasm, and in any direction. In *Bursaria* there is no such regularity in the course of the food vacuole as has been described for *Paramecium* by Nirenstein ('05); and for *Carchesium* by Greenwood ('94). Often digestion and resorption begin and are completed in one and the same place, without any circulation of the vacuole. Residues are extruded from a small area on the dorsal side of the body.

The first visible change which takes place is the absorption of the liquid which has been enclosed with the grain during the

formation of the vacuole. This is always definite and can generally be readily observed and followed throughout the process. This is exemplified by the following experiment:

Experiment II. Single normal individuals which had been placed in tap water 24 hours previously, were each fed a single grain of fresh yolk and the time interval noted between the separation of the vacuole from the base of the gullet and the point of complete disappearance of the liquid about the yolk grain.

Table 1 shows records from ten individuals each fed one grain. From the table it will be seen that the duration of the process is relatively uniform. The point when all the liquid about the grain has been absorbed was determined to within about thirty seconds.

TABLE I
Experiment II

INDIVIDUAL NUMBER	1	2	3	4	5	6	7	8	9	10	AVERAGE
Number of minutes for resorption of liquid about yolk grain.....	4	3½	3	5	5	4½	7	4	3½	5	4.45

When more than one grain is enclosed in the vacuole the same process of absorption takes place, following the separation of the vacuole from the base of the gullet; the vacuole membrane becoming closely applied to the surface of the grains as is shown in figure 2.

Different vacuoles in the same cell are quite independent as to the absorption of fluid. To illustrate, figure 3 is given. Here the new vacuole contained a comparatively large amount of liquid about the grain (in most cases the quantity of liquid is less), and came to be located close to an older vacuole in which the process of digestion of a Paramecium had practically been completed, and which contained a considerable quantity of liquid. The vacuole containing the digested Paramecium was unaffected in size by the absorption of the liquid about the yolk grain in the newly formed vacuole.

An explanation on the basis of osmotic relations within the cytoplasm and in the vacuoles, assuming the vacuole membrane

to remain in a uniform condition, might perhaps account for the visible difference in the vacuoles in this and similar cases. But a conception of this process may more properly be obtained if we

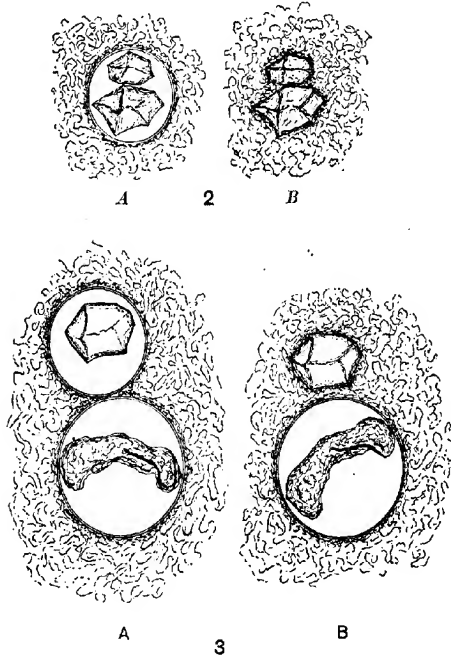


Fig. 2 Shows the resorption of the liquid included with the yolk grains during formation of the vacuole. *A*, food vacuole just separated from base of gullet; *B*, the same, five minutes after separation.

Fig. 3 Showing the independence of vacuoles with respect to resorption of liquid contents. The larger vacuole containing a partially digested *Paramecium* is not affected by the resorption of the fluid in the vacuole containing the fresh yolk grain. *B* drawn 5½ minutes after *A*.

think of it as a process of imbibition of the liquid by the colloidal cytoplasm, accompanied by changes in the permeability of the vacuole membrane. The problem here must be similar to that

which will confront us later regarding the absorption of the liquefied products of digestion.

Within one or two hours after the grain comes to lie in the vacuole, the beginning of solution becomes apparent as a liquefaction and consequent rounding off of the corners and edges. This proceeds in exactly the same manner as when cubes of coagulated egg-albumen are digested by the action of pepsin-hydrochloric acid. At the end of digestion no solid remains of the vitellin grain can be seen; all that is left is more or less liquid in the vacuole.

Another important visible change in the vacuole consists in the usual *second* appearance of liquid about the vitellin grain. It seems most in conformity with the observed facts to consider this as a consequence of the formation of soluble products of digestion, and as being due to the fact that the rate of resorption of these liquid products is less than the rate at which the liquefaction of the vitellin grain takes place.

The reason for this conclusion is partly based upon the fact that in some cases the whole process of digestion of the vitellin may go on and be completed without the appearance of any visible liquid between the grain undergoing solution, and the vacuole membrane. In this case it is obvious that the rate or power of resorption is equal to or greater than the rate of solution of the grain. Furthermore, it is to be expected that as digestion of the protein continues, the affective osmotic concentration of the cleavage products increases and this obviously may bring about an increase in the liquid contents, provided that the permeability of the vacuole membrane (resorption) does not undergo a simultaneous, proportional increase. In other words, the absence or presence of liquid during digestion of vitellin is a resultant of two sets of conditions (a) the rate of digestion and (b) the rate or power of resorption, one factor of which is the degree of permeability of the vacuole membrane. This further agrees with the fact that digestion of a vitellin grain is not always at a uniform rate. A rapid solution of the grain sometimes begins shortly after eating, changing later to a slower one. Even

food vacuoles formed at the same time, of the same material, and in the same individual, may behave diversely as to the resorption of liquid, and also in rate at which digestion takes place. But this individuality of behavior in different vacuoles does not prevent the conception of equilibrium from being applied to the phenomena of resorption. Essentially the same observable physical changes take place in vacuoles containing fresh yolk as has been described for vitellin. Further evidence for this view will be given on page 25.

2. Chemical changes in the food vacuole

Some of the chemical conditions in the food vacuole of *Bursaria* during digestion of vitellin can be shown by the use of sensitive indicators adsorbed by the grains.

Vitellin or fresh yolk grains stained with neutral red remain bright red during the whole process of digestion. Grains stained in an alkaline alcoholic solution of alizarin, quickly lose the blue color and remain colorless throughout the digestive process. Similarly, grains stained in an aqueous blue litmus solution change to red and remain red until nothing remains of the grains. These three indicators agree therefore in showing that the whole process of digestion of vitellin takes place in an acid medium, and that during no part of the process does alkalinity appear, as it does in food vacuoles of *Parmecium* and some other ciliates.

Grains of vitellin or yolk stained with congo red become dark brown after one, two or more hours, and continue to remain dark brown throughout digestion. In some grains no change in color from red to brown takes place, but what differences in conditions account for this has not been determined. Two other indicators were used, Tropaeolin OO and diethylaminoazobenzene. Vitellin or fresh yolk stained with Tropaeolin OO showed little or no change in color. Diethylaminoazobenzene likewise showed no change. This result is due to the fact that these two indicators are not sensitive to very weak acids or strong acids in high dilution.

The points of origin of acid as well as the rate of acidification of the vacuole contents was determined. Table 2 gives typical records of the total time for completely acidifying grains in fifteen out of fifty individuals which were fed with fresh yolk stained in blue litmus. No noticeable difference existed between the acidification of fresh yolk and that of vitellin. The time in seconds which it took to change completely the blue litmus-stained grain to red, is given by the numbers underneath the number of the individual. The average of the observations in table 2 is $38 \pm$ seconds.

TABLE 2

Total time in seconds for acidifying fresh yolk grains stained in blue litmus. The table shows typical records of fifteen out of fifty individuals in which the time for acidification of each grain was taken. The grains which are represented by the numbers opposite to the brackets were included in the same vacuole. The other numbers, not opposite brackets, represent grains, each of which were in a separate vacuole, i.e., swallowed separately.

	INDIVIDUAL NUMBER														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1st grain.....	15	20	{ 10	25	20	65	45	60	80	45	15	{ 30	{ 45	{ 35	55
2d grain.....	35		{ 30	20	15	40	40	10	30	20	30	{ 30	{ 60	{ 35	30
3d grain.....	50		{ 55	15	80		50	20	30			{ 40	{ 50	{ 35	25
4th grain.....			40	15				25	60			45	40	35	
5th grain.....			80	20									60	30	
6th grain.....			30	30										45	
7th grain.....				20										75	
8th grain.....				180										30	

It was evident that the time of acidification depended mainly if not solely upon three conditions, as follows: (a) the size of the grain; the larger the grain, other things being equal, the longer it took for the last trace of blue color to disappear. For example; grain number 8 of individual number 5, table 2, was a very large grain; while grain 2 of individual number 8 was very small, hence the difference in time. (b) The physical consistency of the grain must be assumed to determine its permeability and hence its time of acidification. (c) The concentration of the acid secreted into the vacuole.

It has so far not been possible to determine the relation between the number of grains eaten and the time of acidification of each, because litmus is slightly toxic to *Bursaria* and hence stained grains of even fresh yolk are not eaten very readily.

The arrow at *a* in figure 4 shows the point at which the acid first begins to be secreted into the forming vacuole. This is indicated by the sudden appearance of a faint pink color about the edge of the grain which gradually increases. Figure A in plate 1 shows the degree of acidity reached before the grain leaves the gullet.

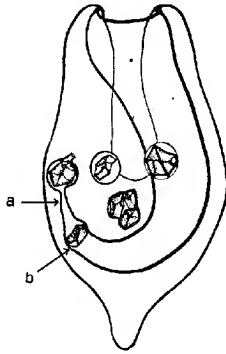


Fig. 4 Outline drawing of *Bursaria* showing steps in the formation of food vacuoles and resorption of the liquid enclosed; *a*, place at which acid secretion begins. Grains of relative size shown will not as a rule be rejected after they have passed the point indicated by *b*.

As the grain passes through the mouth surrounded by the mixture of the acid and the external medium, the change toward red progresses rapidly until the grain's interior has been reached by the acid (plate 1, figs. A, B and C). When the acid had reached the center of the grain the times noted in table 2 were taken. There is no way of telling whether acid is continuously poured into the vacuole after it has left the end of the gullet. No evidence was obtained as to the nature of the acid secreted, nor as to whether it is in combination or in the free condition.

The physical and chemical changes taking place in the vacuole containing vitellin or fresh yolk grains, which have been described above, are briefly summarized in plate 1. Here the series of figures from A to L, inclusive (series 1 and 2), show the usual course of the process of digestion and resorption of fresh yolk when the latter is retained throughout the process. Series 1 and 3 show the usual course of digestion and resorption of vitellin, when the rate of resorption is less than or equal to the rate of digestion. Series 1 and 4 show the same when the rate or power of resorption is equal to or greater than the rate of digestion of the vitellin grain. The successive figures do not represent the condition at equal intervals of time, but are typical stages in the process. Frequently there occurs an alternate presence and absence of liquid or smaller variations in the quantity of liquid around the grain during the stages from H to L, inclusive, and from H' or H'' to L' or L'', inclusive. Thus the figures from H, H' and H'' to L, L' and L'' respectively, represent this process only as it proceeds typically in the majority of cases.

3. *Effect of quantity of vitellin eaten upon the average velocity of digestion*

Since each cell could be fed just the desired number of grains of practically uniform size, the effect of the quantity of protein upon the average rate of digestion, and upon certain other processes could be determined.

Experiments arranged to find out what the relation is between rate of digestion and the quantity eaten, were tried with both fresh yolk and vitellin. Fresh yolk was found unsatisfactory for this purpose, since the grains were generally not retained but extruded before digestion was completed. This was especially true when a large quantity of fresh yolk had been eaten (cf. Experiment VIII, p. 34). Under usual conditions vitellin fed to the animals was not extruded, and this was especially true if care was taken not to let the animals eat too many grains. The average limit in the number of grains that could be eaten and retained was determined from a large number of observations

during the work. In these it was found that when normal, active animals were fed vitellin under what seemed to be the best experimental conditions, five to seven or even nine grains were retained, and digestion of the whole mass continued to completion, leaving no residues.

In the experiments, to determine the average rate of digestion of vitellin the maximum number of grains fed was six, and it was found that with this number as the maximum extrusion very rarely took place.

Rise in temperature accelerates the digestive process, but the variations due to temperature were eliminated, since all the sets of individuals were side by side in an oven kept at 25 to 26°C., and the fluctuations in temperature due to taking the moist chambers from the oven while the records were taken, were the same for each set of individuals.

The organisms used in each experiment were from the same healthy culture. They were starved in tap water twenty-four hours previous to the beginning of the experiments, so that a clear cell was obtained.

When the animals were to be fed, a large number were removed from the 500 cc. dish of tap water in which they had been starved, to an 8 cc. dish containing tap water. Some of the vitellin suspension prepared as described above (p. 3), was added to the tap water in the dish containing the animals. The quantity of vitellin suspension added varied according to whether it was desired to feed a small or large number of grains to each individual. If a large number of individuals were to be fed only one grain, a very weak suspension was added; the result was that the rate of feeding was slow, and hence gave sufficient time to remove the individuals as soon as one grain had been swallowed. As feeding went on, the individuals that had eaten the desired number of grains were picked out with a pipette and placed in separate dishes containing tap water. In this way, under favorable conditions, it was possible to obtain several sets of individuals simultaneously, each individual of each set having eaten a definite number of protein grains. After feeding, the animals were

removed from the dishes with tap water, and two individuals were placed in each watch glass in order to lessen the labor in taking the records. Each watch glass contained 2 cc. of tap water. The watch glasses were placed in moist chambers. In order to avoid the effect of individual variations in the rate of digestion of equal numbers of grains, forty-eight individuals were used in each set. Effects upon the result due to variation in size of the grains were therefore also practically avoided. Digestion was taken to be complete when all the solid contents of the vacuole had become liquefied. The results of two experiments are given.

Experiment III, February 12, 1913: Table 3. Each individual of Set A, Set B and Set C was fed 1, 3 and 6 grains respectively. Examination of each individual was made and records taken, $3\frac{1}{2}$, 5, $6\frac{1}{2}$, 8, $9\frac{1}{2}$, 11, $12\frac{1}{2}$, 14 and 22 hours from the time of feeding.

The rate of digestion and resorption when a large number of grains have been fed is in many cases slow toward the end of the process; so that it is sometimes difficult to tell at just what time (within 1 or $1\frac{1}{2}$ hours) the last remains of solid protein disappear. For this reason eighteen hours was taken as the average time of complete digestion in those individuals of Set C which had small protein residues at the end of fourteen hours and none at twenty-two hours. All of Set C showed complete digestion at the end of twenty-two hours.

In each of the three sets (table 3) individuals numbered 2 show a longer time for digestion than their partners numbered 1. This has no significance, because it is due to arbitrarily calling the first individual that had completed digestion, number 1, and the last one, number 2; when the record was taken. The same is true for table 4, Experiment IV.

It will be seen from table 3 that the average number of hours which it takes to complete digestion in the three sets A, B and C, is not directly proportional to the number of grains eaten, i.e., as 1:3:6. Before further consideration of these results the next experiment will be described.

TABLE 3
Experiment III: February 12, 1913—Number of hours for complete digestion
 Set A—Forty-eight individuals, each fed 1 grain of vitellin

WATCH GLASS NO.																								TOTAL HOURS TO COMPLETE DIGESTION	AVERAGE NUMBER HOURS TO COMPLETE DIGESTION
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24		
Set A—Forty-eight individuals, each fed 1 grain of vitellin																								339	7.06
Individual No. 1.....																									
Individual No. 2.....																									
Set B—Forty-eight individuals, each fed 3 grains of vitellin																								439.5	9.15+
Individual No. 1.....																									
Individual No. 2.....																									
Set C—Forty-eight individuals, each fed 6 grains of vitellin																								726	15.12+
Individual No. 1.....																									
Individual No. 2.....																									

TABLE 4
Experiment IV: February 18, 1918—Number of hours for complete digestion
Set A—Forty-eight individuals, each fed 1 grain of vitellin

	WATCH GLASS NO.																								AVERAGE NUMBER OF HOURS TO COMPLETE DIGESTION	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24		
Individual No. 1.....	8	9½	9½	6½	5	8	8	5	8	8	6½	8	8	6½	8	5	8	9½	9½	9½	8	5	8	8	411	
Individual No. 2.....	8	11	11	8	6½	8	9½	11	11	8	9½	8	9½	8	8	6½	9½	9½	12½	11	9½	11½	8	8		
Set B—Forty-eight individuals, each fed 2 grains of vitellin																										
Individual No. 1.....	12½	9½	11	9½	12½	8	8	11	11	6½	8	9½	6½	9½	6½	9½	9½	9½	8	14½	2½	9½	8	9½	8	527
Individual No. 2.....	15½	11	14½	18	14	12½	15½	12½	15½	15½	14	9½	8	15½	12½	11	9½	15½	17½	14	14	8	12½	17	8	
Set C—Forty-eight individuals, each fed 3 grains of vitellin																										
Individual No. 1.....	15½	14	14	9½	9½	12½	11	11	6½	9½	9½	8	8½	8	12½	9½	11	11	9½	13½	15½	12½	8			612
Individual No. 2.....	17	15½	18½	11	17	18½	12½	18½	12½	17	13½	18½	11½	15½	9½	14	9½	15½	11	9½	18½	17	9½			
Set D—Forty-eight individuals, each fed 6 grains of vitellin; four marked X omitted from calculations because of partial extrusion of vitellin																										
Individual No. 1.....					15½	15½	14	8	15½	18½	14	12½	15½	14	14	17	9½	14	9½	14	17	18½	17	17		893
Individual No. 2.....					15½	22½	22½	20	22½	18½	22½	22½	22½	22½	20	22½	22½	20	20	22½	22½	22½	22½	22½		

Experiment IV: Table 4. In this the procedure was the same as in the previous experiment, but the animals were taken from a different culture, and instead of 3 sets of 48 individuals each, 4 sets of 48 individuals each were used. Each individual of Sets A, B, C and D were fed 1, 2, 3 and 6 grains, respectively.

Four individuals (marked X in the table) of Set C extruded part of the six grains and are therefore not counted in the results. The temperature was 25 to 26°C. Examinations were made $3\frac{1}{2}$, 5, $6\frac{1}{2}$, 8, $9\frac{1}{2}$, 11, $12\frac{1}{2}$, 14, $15\frac{1}{2}$, 17, $18\frac{1}{2}$, 20 and $22\frac{1}{2}$ hours after feeding. In this experiment the possible slight error in the average time for complete digestion in Set C, Experiment III, due to the approximation of the time of complete digestion in some of these individuals, is eliminated, because observations were made regularly throughout the whole $22\frac{1}{2}$ hours. And the conditions were on the whole better than in Experiment III.

The results of both experiments agree in showing that the average total amount of vitellin digested per unit of time is greater in individuals fed a larger quantity than in those fed a smaller one, i.e., this quantity is greatest in Set D > Set C > Set B > Set A.

The average time, found by experiment, for the complete digestion of one grain or its equivalent in volume or mass, in the sets of individuals of the preceding two experiments is given in column 2 of table 5.

Now if we establish a limiting case, by supposing that the average quantity of vitellin digested per unit of time, is a function only of the amount of surface of the substrate exposed to the action of the digestive agent, then we should expect to find (provided further that the total surface of each grain in the individuals fed more than one grain was exposed to the action of the digestive agent), that individuals fed 6, 3 and 2 grains each, would complete digestion of all in the same length of time as it takes to digest one grain. Hence, if it takes 8.56 hours (Experiment IV) to digest one grain, then in Sets B, C and D we should expect to find that the quantity of vitellin equal to the volume of 1 grain, would undergo digestion in the times shown in table 5, column 3.

The results are uniformly *less* than those found by experiment (column 2).

Now since we have constructed a limiting case for the maximum average rate of digestion on the assumption that the rate is in direct proportion to the surface of substrate exposed, we may on the same assumption establish a limiting case for the minimum average rate of digestion, by assuming that the surfaces exposed to digestive action in the four sets, are to each other as the surfaces of spheres of vitellin having volumes 1, 2, 3 and 6. Calculating this on the basis of the rate of digestion found by experiment for one grain (Set A), we obtain the values given in column 4, table 5. It will be noted that these values, based on the above assumption are all *greater* than the values found by experiment. The results of both experiments agree. However, the intermediate position of the obtained results (column 2, table 5) does not exclude the possibility that the digestive process

TABLE 5

Summary of and calculations for the average velocity of digestion of vitellin, based on results of Experiments III and IV, tables 3 and 4

EXPERIMENT	SET	COLUMN					6
		1	2	3	4	5	
		Number grains fed to each individual	Average time found for complete digestion of one grain, or its equivalent	Calculated average time for complete digestion of one grain or its equivalent, on basis of surface, grains separate	Calculated average time for complete digestion of one grain or its equivalent, on basis of surface, total number of grains a sphere	Average time for complete digestion of one grain or its equivalent, calculated from $t = k\sqrt{N}$	Value of k in $t = k\sqrt{N}$
			hours	hours	hours	hours	
III. February 12, 1913	Set A...	1	7.06				7.06
	Set B...	3	3.05	2.35	4.89	4.06	5.28(?)
	Set C...	6	2.52	1.17	3.88	2.846	6.17
IV. February 18, 1913	Set A...	1	8.56				8.56
	Set B...	2	5.62	4.28	6.76	6.04	7.96
	Set C...	3	4.25	2.85	5.93	4.94	7.38
	Set D...	6	3.04	1.42	4.70	3.49	7.44

really conforms to the law of a heterogeneous chemical reaction (between a solid and a liquid).¹

The values in column 5, table 5, were obtained by substitution in Arrhenius' formula $t = k\sqrt{M}$ for the rate of digestion in the dog as found by London.² The values obtained by means of this formula agree well, within the limits of experimental error, with those found by experiment in the case of *Bursaria*.

In column 6, table 5, are given the values of k in the formula $t = k\sqrt{M}$. The agreement is not so good in Experiment III as in Experiment IV for reasons given above, although the variation in the constant k of both experiments, and especially that of Experiment IV, is well within the limits of experimental error.

If it were possible, it would be interesting to determine what the value of k would be for other food substances.

The experiments show that in spite of individual variations among the organisms and the variation in the process in different vacuoles in the same individual which were mentioned above, the sum total gives a definite result, and shows that the average rate of digestion of vitellin under rigid experimental conditions follows a definite law.

4. *Effect of congo red upon the average velocity of digestion, extrusion, etc.*

In order to discover some of the changes which take place in the reactions of the cell when protein (vitellin grains) was changed by letting it adsorb a substance from solution, the following experiment was carried out. Congo red was chosen since its toxicity to *Bursaria* is low when compared to that of many dyes,

¹ The intermediate position of the values found suggests agreement with the results of the experiments of Bayliss ('01), who found that "with concentrations of casein up to about 4 per cent, the velocity of digestion is proportional to the concentration of the substrate . . . whilst with more than 8 per cent, inverse proportionality sets in" (cited from Euler: General chemistry of the Enzymes, p. 186). Furthermore the results also suggest the possibility that the quantity of active agent produced in the cell or vacuole is not directly proportional to the quantity of vitellin eaten.

² This is, $t = k\sqrt{M}$; where t is the time for complete digestion, M the amount by weight eaten, and k a constant depending upon the nature of the food.

and since vitellin grains readily adsorb it in considerable quantity, depending upon the length of time during which the grains are left in the aqueous solution of the dye.

The results of the experiment clearly show: (a) that the adsorbed congo red very markedly interferes with or prevents digestion of the parts of the vitellin grain to which the congo red has been adsorbed, and (b) that it brings about a condition which leads sooner or later to an extrusion of the contents of the vacuole; (c) that it may exert a greater or less toxic effect from within the vacuole, upon the cell, which may lead to an earlier death than if the unstained vitellin had been eaten. In short: *the chemical nature of the substance taken into the vacuole of Bursaria may determine in various ways what many of the conditions and reactions of these organisms will be*, and especially, in this connection, the action of the digestive agent and the process of extrusion.³ The following are the results in brief.

Experiment VI. Grains of vitellin of uniform size, prepared as described above, were stained twenty minutes in a deep red aqueous solution of congo red. They were then washed several times until no more stain could be washed out. An unstained portion of the same vitellin sample was washed the same number of times in tap water; this was fed to the control individuals. Forty normal individuals previously starved in tap water for 24 hours were each fed one grain of the stained vitellin. Similarly a control set of 40 individuals were fed, each one grain of the unstained vitellin. All the conditions and material were the same in the two sets, except that the individuals of one set was fed stained grains, those of the other set unstained ones. Both sets of individuals were kept in watch glasses, each containing 4 cc. of spring water. Two individuals were placed in each watch glass. Records of each individual were taken 2, 4, 6, 7, 9, 11, 13 and 22 hours after feeding.

Table 6 gives the results. Table 6 (a) shows that the partially digested or undigested congo red stained grains were to a large extent extruded, while in the control set, all retained the unstained

³ I have here used the term 'chemical nature,' and in my previous paper (Lund '14) such terms as 'toxicity' (p. 29), 'specific chemical properties' (p. 41), with a general meaning. The finer distinction between the effects due to chemical and physical properties of a substance, as for example its solubility in the plasma membranes, state of colloidal aggregation, chemical reaction with the protoplasm, etc., remains an open question.

protein grains. This, therefore, shows that the *chemical* nature of the substance in the vacuole may, and does in this case, determine whether it shall be extruded or not. Table 6 (b) shows the effect of the adsorbed dye upon the process of digestion of vitellin. The stain retards and sometimes even prevents digestive action. This was seen from the fact that the corners and edges of the grains where little or no unstained yolk exists, persist more markedly than those of the unstained grains. The stained grains, nevertheless, often became smaller by shrinkage, caused apparently by solution of the protein from the deeper parts of the vitellin grain, which may have been stained less deeply. That the adsorbed dye interferes, to some extent, with the maintenance of the normal form and outline of the animal is shown by the results of table 6 (c). The fact that, for example, the total number of normal individuals of those fed a stained grain has increased rather than decreased at the eleven-hour record, since the nine-hour record was taken, is due to regulation of form, either by closing and opening the oral pouch, or by undergoing

TABLE 6

Showing the results of feeding one grain of vitellin stained in congo red, to each of a set of forty individuals. For comparison (control) another set of forty individuals were each fed 1 grain of unstained vitellin

		TIME IN HOURS AFTER FEEDING								
		2	4	6	7	9	11	13	22	
(a) Total number of individuals that had extruded grains before death	Stained.....	0	0	1	4	9	18	25	27	
	Unstained.....	0	0	0	0	0	0	0	0	
(b) Total number of individuals that had completed digestion of grains	Stained.....	0	0	0	0	0	0	0	0	
	Unstained.....	0	0	1	4	10	22	28	37	
(c) Total number of individuals that were normal in form	Stained.....	38	35	27	26	15	24	23	14	
	Unstained.....	37	38	37	38	34	33	35	29	
(d) Total number of individuals dead	Stained.....	0	0	0	0	0	3	4	11	
	Unstained.....	0	0	0	0	0	0	0	2	
(e) Total number of individuals with a perceptible amount of liquid in vacuole	Stained.....	0	0	0	0	3	2	0	0	
	Unstained.....	0	4	7	13	13	16	3	2	

more fundamental morphological changes. A full consideration of these processes will be given at a later time.

That the presence of the dye also brings about a tendency to an earlier death of the individual is indicated by table 6 (d). The record for this is not complete because when the experiment was stopped a large number of the individuals still remained alive, as the table indicates.

It was stated above (p. 11; cf. figs. H, H' and H'' to L, L' and L'' respectively, plate 1) that the rate of resorption during digestion of vitellin may be equal to, or less than, the rate of solution of the grain. This is a definite and observable condition, for in some cases solution of the grain may go on with the vacuole membrane closely applied to the grain, while in other instances, even in a sister vacuole in the same cell, the process of dissolution of the grain may go on while the latter is floating in the liquid in the vacuole, which is made up, in part at least, of the liquid products of digestion. The quantity of liquid present in such vacuoles during digestion, may vary, and when only a smaller quantity is present around the grain, it becomes more difficult to observe its presence; but, when the quantity is sufficient to be seen, then records may readily be taken giving (practically) the absence, or presence of liquid about the grain. The results from Experiment VI are given in table 6 (e).

It is evident that there was a much greater tendency toward accumulation of liquid about the unstained grain than in the vacuole having the stained grain. In other words, *along with decrease in digestive action upon the stained grains went a strong tendency toward absence of liquid in the vacuole.* We have therefore in this result further good evidence for the essential correctness of the statement made above (p. 11) that the rate of digestion of the vitellin may be (1) greater, (2) equal to, or (3) less than the rate or power of resorption.

Further evidence for this is shown in table 6 (e), for *during the height of digestive activity, which in this case occurred between six and eleven hours, the tendency toward presence of liquid in the vacuole is greatest.*

It is not to be supposed that the appearance of liquid in food vacuoles generally, under other conditions, need necessarily be due to rapid digestion and slow resorption (cf. fig. 3). In such cases we may have other factors bringing about accumulation of liquid.

Other tests of the effect of congo red upon digestion gave similar results, but since records of individuals were not taken these results are not expressible quantitatively.

DIGESTION AND RESORPTION OF FAT

1. *A demonstration of fat digestion and resorption in the food vacuole of Bursaria*

Nirenstein ('10) and Issel have furnished a simple and reliable method for determining the presence of lipoids in infusoria.⁴ The method consists simply in placing a small drop of the culture fluid containing the Infusoria on a slide and killing and staining by addition of a few drops of an alcoholic solution of Sudan III. The mount is cleared with NH_4OH followed by thick glycerine. By proper manipulation beautiful mounts may be obtained. The gross distribution of lipoid in the cell is faithfully preserved by this method. This is shown by the fact that fat globules of the same typical sizes as in the mounts may readily be distinguished in the ground substance of the living protoplasm and followed during protoplasmic movement especially in individuals with high fat content. Bursariae taken directly from healthy cultures always had more or less fat present in the protoplasm. This fat is probably derived from other Protozoa which serve as food.

When an individual from a wild culture, with a high fat content, is isolated and placed in tap water, the protoplasm usually becomes clearer after one or more days; showing that fat, as fat droplets at least, disappears from the cell.

The chief reason for the difference in results and conclusions of Nirenstein ('10) and Staniewicz ('10), as to whether Infusoria,

⁴ The words 'lipoid' and 'fat' are used here to designate mainly those fat-like substances in the cell which are amenable to detection by microchemical tests.

particularly *Paramecium*, actually do digest, resorb, and utilize fat for the energy requirements of the cell, appears to be the fact that these investigators did not compare the fat contents of the *same* cells nor *sister* cells, before and after feeding of fat, but limited their comparison to different individuals taken at random from the same culture.

In order to leave absolutely no question as to the fact of digestion and resorption of fat in *Bursaria*, cells which were in the process of division were isolated from the wild cultures. Those which had many vacuoles were discarded, and in most cases only those with no food vacuoles were used. Each pair was placed in a separate watch glass with tap water. After the completion of division, or after eighteen to twenty-four hours' starvation in the tap water, one of the sister cells was fed an emulsion of olein or pure olive oil (both gave the same results), while the other sister cell was kept as a control in a separate watch glass with tap water, under exactly the same conditions.

In different experiments, at different intervals after feeding, both sister cells were tested simultaneously for the amount and distribution of the fat content. In every experiment, after a sufficient length of time, the cell which had eaten and retained the olive oil or olein for some time showed an abundance of fat droplets distributed throughout the cytoplasm, while the remaining fat in the vacuoles appeared as large, single fat droplets or as groups of such.

The fat content of food vacuoles can of course be readily seen and followed in the living cells. In the control (sister) cell a much smaller quantity of fat was invariably present, this quantity depending upon the original amount present in the cell before isolation.

That the fat is distributed equally to sister cells in dividing individuals which have no food vacuoles at the time of isolation, was of course tested by mounting sister cells and comparing the fat contents.

The result of a typical experiment is given by the camera lucida drawings. Plate 2, figure A, shows the control with a small fat content twenty-four hours after the time when its sister

cell (fig. B) had been fed olive oil. Figure B shows the sister cell twenty-four hours after feeding with olive oil. The large fat drop in B constituted the contents of the food vacuole previous to mounting. Both cells were starved in tap water for eighteen hours previous to feeding of sister cell (fig. B). The same results were obtained when fresh yolk grains were fed.

These experiments prove conclusively that in *Bursaria* the fat in the food vacuoles passes out in one form or other through the membrane of the food vacuole, and becomes distributed in the cytoplasm as small droplets. This agrees perfectly with the results obtained by Nirenstein ('10) for *Paramecium*. Liquid may at times be present about the fat droplets undergoing resorption in the vacuole, just as in the case of vitellin or yolk (plate 1, H to L). But no attempt was made to study its changes. Results from a study of the chemical changes involved in digestion and resorption of fat will be given elsewhere.

2. Role of fat in growth and energy requirement

The tail, in the form of *Bursaria* studied, serves in general, as a convenient index of the degree of 'fatness' or 'leanness' of this organism.

When olive oil is fed to a large number of *Bursariae* under favorable experimental conditions, so that more or less resorption takes place, the fed animals, on the average, become larger than the unfed. The tail on the average also assumes more pronounced outlines than those of the control. In such large individuals when in sufficient number for comparison with the controls, there is indication also that the length of life is on the average increased by having eaten olive oil. When individuals fed a definite number of grains (1 or 2) of fresh yolk, are compared with others fed the same number of fat-free yolk grains, the individuals fed with fresh yolk, become on the average larger, many of them attaining a relatively enormous size. During this growth perfect proportions of form are maintained. Such large individuals are always found to contain much fat. The part of the Curve A

(fig. 1, Experiment I, from 144 to 288 hours, represents individuals of this kind and constitutes evidence that the length of life of individuals fed fresh yolk is greater than that of those fed fat free yolk. During this time the quantity of fat in the cell decreases as starvation continues, indicating that the fat is used up.

Under some conditions no traces of fat can be found in Bursaria by the use of Sudan III, but this of course does not prove its complete absence. The apparent absence of fat is rare and it was found difficult by means of starvation to remove the last traces.

3. *Is fat formed from vitellin or starch in Bursaria?*

When yolk is extracted repeatedly with cold ether or with cold alcohol, at intervals, for twenty-four hours or more, a white residue remains, which is chiefly vitellin. Such vitellin was fed to Bursariae which had been starved. An abundant fat deposit appeared in the cytoplasm, indicating that yolk extracted with cold ether or alcohol alone still contained a fraction which appeared as fat in the cells. This was tested by extracting fresh hard boiled yolk with ether-alcohol mixture for ten to twelve hours in a Soxhlet apparatus. Vitellin so extracted was fed to one of each pair of sister cells while the other cell was kept as control. No difference appeared in the amount of fat present in the two cells, although many attempts to demonstrate a difference were made.

In view of the fact that crucial control tests were not made by Nirenstein ('10) the slight difference in fat content given in his figures 7 and 1, plate 1, cannot I think, be considered proof that the fat found to be present in *Paramecium* (fig. 7) after digestion of egg albumen, had been derived from the latter; furthermore, analyses of egg albumen show traces of the presence of lipoids, such as fat, cholesterin and lecithin.

• Various kinds of starch and paramylum grains which were retained by Bursaria, remained intact for days and showed no signs of corrosion or solution.

THE NATURE OF SOME OF THE FACTORS WHICH BRING ABOUT
EXTRUSION*1. Experiments with paraffin oil and olive oil*

If a conception can be obtained of what some of the dynamic conditions of the cell are, by a study of the processes of intake of foods and other substances, then we might expect to find that the process of extrusion will serve as a valuable index in discovering what some of the responses of the cell system are to those same substances, after they have produced their effects from the interior of the cell. Relations as interesting as those discovered in feeding ought to be found by studying the phenomena of extrusion, though the process, for various reasons, does not lend itself so readily to experimental inquiry.

I have shown (Lund '14) that when *Bursaria* has been fed Sudan III or Chinese ink alone, these substances are not retained within the cell for any considerable length of time, and further that if *Bursaria* is fed at the same time one of these substances and vitellin, then the Sudan III or Chinese ink is extruded after a short time, provided it has not come to be located in the same vacuoles which contain vitellin; while the vitellin is retained, digested and resorbed. In other words there exists a selective extrusion among food vacuoles formed at the same time, or different times. Above (p. 24, table 6, a) it was further shown that in the case of vitellin grains stained with congo red, a large number were extruded during digestion, or before any digestion had taken place, while unstained grains were retained and digested by the control individuals. Here the condition which brought about extrusion can be only the presence of the absorbed dye, i.e., a chemical condition. Since both sets of individuals were fed one grain each, the size or shape of the grain had nothing to do with the difference between the results.

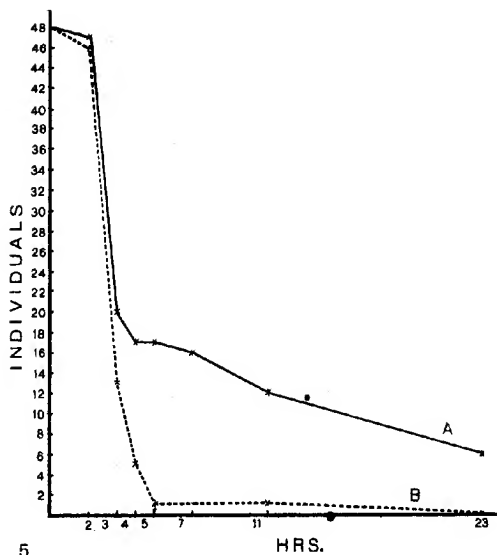
When both unstained grains, and grains stained with congo red, were fed to the same individuals, similar results were obtained. Further to test this by feeding two liquid substances of somewhat similar physical properties, but of different chemical nature, the following experiment was carried out.

Experiment VII, a, b and c: (figs. 5, 6 and 7). Emulsions of paraffin oil, and olive oil were made in tap water. The animals were starved as usual in tap water. 2 cc. of this tap water was placed in each watch glass and used as a medium, so as to prevent any effect due to transfer from this into fresh tap water. Set A consisting of 48 individuals that had eaten olive oil were picked out from the 8 cc. with olive oil emulsion in which they had been feeding for 10 minutes. Another set, B, of 48 individuals that had been fed an emulsion of paraffin oil were picked out at the same time. Both sets of individuals were washed in some of the tap water in which they had been starved. After washing, two individuals were placed in each watch glass, the latter being kept in moist chambers. The moist chambers were placed in a constant temperature oven kept at 25 to 26°C.—an unnecessary precaution for the purpose of the experiment. Records were taken at the time intervals given by the points on abscissae in the curves.

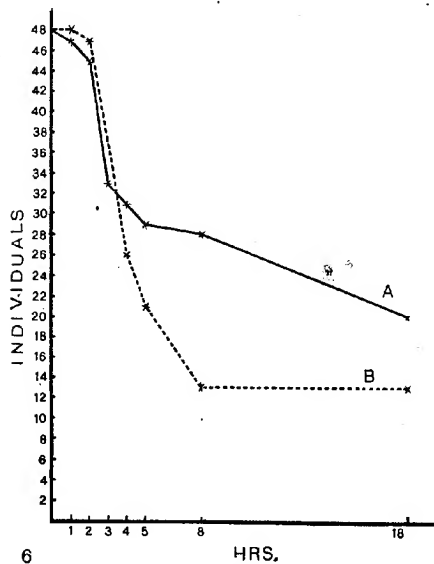
The process of extrusion was gradual, i.e., all the paraffin oil present in an individual was not usually extruded at the same time. Small globules or traces generally remained for various lengths of time after the main bulk of the oil had been extruded. Since there was no way of expressing the course of this process quantitatively, when oil was used, the time when all trace of oil in the food vacuoles had disappeared was taken as the time of extrusion, for comparison in the two sets of individuals, A and B. Hence the curves are based on the time for complete extrusion. As a matter of fact, the difference in the time of extrusion between Set A and Set B was actually greater than the curves show, for the bulk of the paraffin oil was extruded, on the average, considerably earlier than that of the olive oil.

The experiment was carried out three times, all giving concordant results, as shown by curves, figures 5, 6 and 7. In no individual in the above experiments was the disappearance of olive oil due to retention and complete digestion. Fats are resorbed very slowly. The material came from three different wild cultures, otherwise the procedure was the same in all. The extrusion reaction was apparently not as effective in Experiment VII, b (fig. 6) as in the others.

Tests for fat content showed marked resorption in those individuals which had retained some or all of the olive oil for some time after feeding. No increase in the fat (oil) content could be seen to have taken place in individuals of Sets B fed paraffin oil.

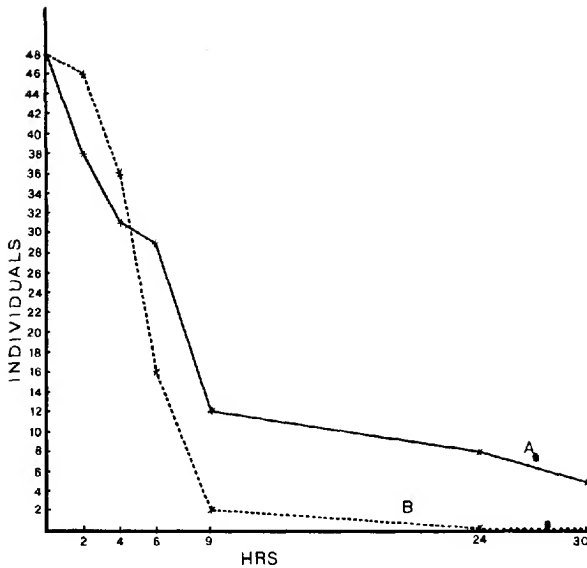


5



6

Figs. 5, 6 and 7 Curves of three separate experiments (VII, a, b and c) showing the course of complete extrusion among individuals fed olive oil (Curves A), and paraffin oil (Curves B). Points on the ordinates indicate the total number of individuals still containing oil at the time indicated on the abscissae.



7

The Sudan III method serves for the detection of paraffin oil[•] as well, since this stains red also.

These experiments with oils again show that the retention or extrusion depends, at least in part, upon the chemical nature of the substance, and that the food vacuole functioning as an organ of extrusion may vary in its response to qualitatively different chemical stimuli.

Does the extrusion of a substance depend *alone* upon a response to the quality of a chemical stimulus by the vacuole and whatever other part of the cell system it may be specially related to in this response? If there are other factors concerned, what are they?

2. *Effect of mass or volume upon the extrusion reaction*

From previous observations it had become evident that extrusion depended in some way upon the quantity eaten, and accordingly the following experiment was carried out:

Experiment VIII (table 7, fig. 8). Three sets, A, B and C, of 48 individuals each, were fed fresh yolk. Individuals of Set A were fed 1 grain each, those of Set B, 3 grains each, while those of Set C were permitted to eat a considerable number of grains, at least 5 or more. Several of Set C ate 12 to 15 grains. The number of grains present in each individual of Set C was counted when the first record was taken, 3 hours after feeding. Several individuals of Set C had by this time extruded a number of the grains eaten three hours previously.

The count of the number of grains present in each individual of Set C was not made immediately after feeding, because it becomes increasingly difficult to count the number of grains in active individuals that have eaten more than 7 or 8 grains. The average number of grains in each individual of Set C, three hours after feeding, was 5.64. The number of grains in each varied from 4 to 7. The total number in the 48 individuals Set C, at the end of three hours was 271 grains.

Records of the number of grains in each individual of the three sets were taken 3, 5, 7, 9, 12, 14, 16, 24 and 30 hours after feeding. The results are given in brief in table 7, and by the curves in figure 8. The Curves A, B and C show the relative rates of total loss of the fresh yolk by Sets A, B and C, respectively.

The table shows that the total number of grains present in all the individuals of each set, decreases with increase in the length of time after feeding. The rate of decrease in these numbers is on the average in the order: Set C > Set B > Set A. This becomes more apparent if we calculate the percentage of the total number of grains fed which was present in the individuals at the times when the records were taken. In order to bring out these relations more clearly the curves in figure 8 were plotted from the percentages given in table 7.

Now, from what has thus far been said, and from the records, we have no proof that the loss in the number of grains which did

occur was not, in part at least, due to complete digestion and resorption of the missing grains. But this difficulty is almost wholly avoided in this experiment, because the process of digestion and complete resorption of fresh yolk is much slower than that of vitellin alone. This is mainly due to the fact that the fat of the fresh yolk is digested and resorbed at a slower rate than the vitellin of the yolk grain (cf. plate 1, figs. H-L, and figs. H'-L'). This fact is further shown in the records of the total numbers of grains present in Set A, table 7, for twenty individuals out of forty-eight, still contained remains of the *one* grain fed *thirty* hours previously, this should be compared with the average time for digestion of one grain of vitellin in Experiments III and IV, table 5, column 2. Now, from these facts regarding the difference in rates of digestion of one grain of vitellin and fresh yolk, it is not to be expected that the individuals of Sets B and C would show as rapid a rate of digestion as if they had been fed an equal number of grains of vitellin; and therefore it becomes clear that if the comparatively small total number of grains pres-

TABLE 7

Summary of results of Experiment VIII (January 24, 1913) showing the effect of the quantity of fresh yolk eaten, upon the course of the extrusion reaction in Bursaria. Three sets of forty-eight individuals each were used. Each individual in the Sets A, B and C, was fed 1, 3 and 6 grains, respectively

[illegible]

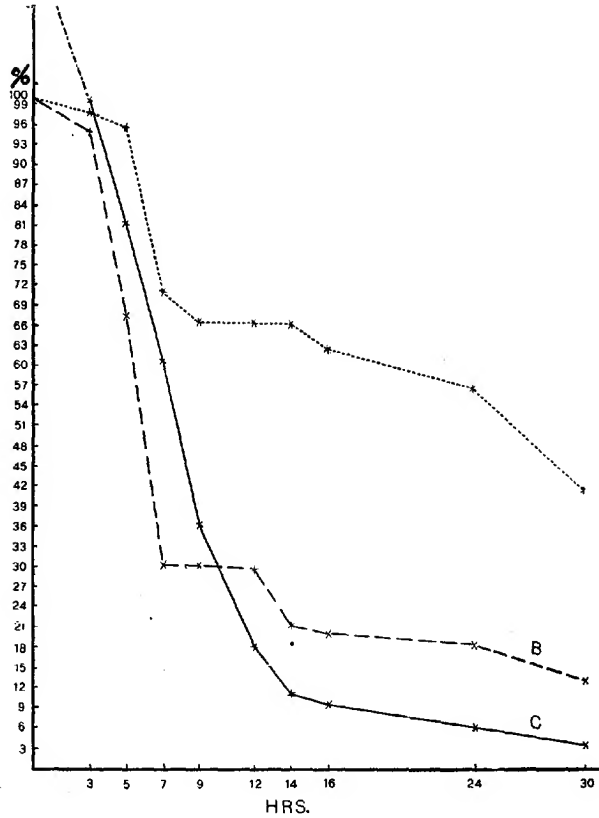


Fig. 8 Curves plotted from percentages in table 7, Experiment VIII, showing the effect of mass or volume of fresh yolk eaten, upon the extrusion reaction. Curves A, B and C are plotted from Sets A, B and C, respectively. The points on the ordinates represent the percentage of total number of grains fed, which still remained in the vacuoles at the time indicated by the same points on the abscissa.

ent, towards the end of the experiment, in the individuals of Sets B and C, was caused by rapid and complete digestion, then the effect of mass on the rate of digestion for fresh yolk would have to be relatively vastly more pronounced with fresh yolk grains than is true for vitellin; and this is not the case.

The first parts of the curves (fig. 8) bring out the points clearly, for here the error, that would be caused by the loss of grains due to complete digestion and not to extrusion, is practically avoided, since the rapid fall in the number of grains takes place during the first part of the experiment, before the digestive process could have been effective in causing total disappearance of the yolk grains; especially is this true for Set C, Curve C. On the other hand, Curve A which we should expect to be markedly affected by digestion, does in fact show only a slight fall, compared to that of Curves B and C. It will be noted that the amounts of fall of the curves show the same *sequence* as the number of grains fed.

The results therefore actually demonstrate beyond question, that the *quantity* of fresh yolk eaten is a determining factor in the process of extrusion. The greater the mass or volume the more effective is the stimulus from the contents in the vacuole. *A quantitative or intensity factor as well as a qualitative factor therefore enters and determines whether or not extrusion of fresh yolk shall take place.*

Now from the results of Experiment VIII we have as yet no evidence showing in what way the mass or volume affects the process; i.e., whether it is an intensity effect due to chemical or mechanical stimulus or both.

Another important fact in connection with the extrusion reaction is that the stimulus from the contents of the vacuole is most effective in bringing about extrusion during a rather limited period (4 to 6 hours with fresh yolk grains) after the formation of the vacuole. This will be seen from the sudden drop in the curves in figure 8, with subsequent tendency to retention of the remains of the yolk. There is a process of functional adjustment (loss of irritability?) with the continued action of the stimulus from the contents of the vacuole. Roughly it may be ex-

pressed by saying that a reaction by digestion is substituted for one by extrusion. These facts appear in a clearer way when the course of extrusion is studied in single individuals.

A further analysis of these responses must be kept for a future time, since the data from other experiments in which were used other substances are not sufficiently complete.

SUMMARY

1. A quantitative method (p. 3) was worked out, by means of which it was possible to study the processes in the food vacuole of *Bursaria*.

2. Yolk and vitellin may be drawn upon as food for the energy requirements and growth of *Bursaria*.

3. The liquid of the newly formed food vacuole is partly made up of the external medium, and partly of an acid secreted by the base of the buccal pouch. After a few minutes this liquid is resorbed and the vacuole membrane becomes applied to the yolk grain. The vacuole contents remain acid in reaction throughout the process of digestion of vitellin and yolk grains.

4. Sooner or later after the initial resorption of liquid about the grain, digestion begins. Digestion may or may not result in the second appearance of liquid in the vacuole, according to the principle that whenever the rate of solution—this perhaps in part depending upon the concentration of the cleavage agent—is greater than the rate of resorption, then the liquid products of digestion accumulate more or less about the grain, while if the rate of solution of the grain is slower than the rate of resorption, then the products of digestion are removed as fast as they are formed. Equilibrium between these processes in the vacuole may be established during digestion of vitellin with much, little, or no liquid present in the vacuole.

5. The average time for complete digestion of vitellin in *Bursaria* was found to be directly proportional to the square root of the quantity of vitellin eaten, i.e., the relation expressed by Arrhenius' formula $t = k\sqrt{M}$ was found to hold to within the limits of experimental error.

6. Congo red adsorbed by vitellin grains and fed to Bursaria interferes with or prevents digestion of the parts of the vitellin grain to which the dye has been adsorbed and causes an early extrusion.

7. Olein is digested and resorbed by Bursaria while paraffin oil is not affected. Lipoids and fats play an important rôle in promoting growth in Bursaria. No evidence was obtained for the formation of stainable lipoid from pure vitellin.

Starch or amyllum grains are not digested.

8. The time of extrusion is determined by the quality (chemical) and the quantity or intensity (chemical, physical or both) of the stimulus from within the vacuole by the substance eaten.

9. The maximum tendency to respond by extrusion to the stimulus from the vacuole contents, exists within a limited time (4 to 6 hours with fresh yolk) after feeding.

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PLATE 1

EXPLANATION OF FIGURES

Typical stages in the process of digestion and resorption in a food vacuole of *Bursaria* containing a single grain of fresh yolk or vitellin.

A Degree of acidity attained by the vacuole as shown by litmus at time of separation from the end of the gullet. The pink edges indicate that acid has been secreted from wall of gullet; figures B and C, further stages in same process.

A to E, inclusive, show course of resorption of liquid enclosed during formation of vacuole.

G to L, inclusive, typical stages of digestion and resorption of fresh yolk (protein and lipoid).

G and H' to L', inclusive, typical stages in digestion and resorption of vitellin-liquid present in the vacuole.

G and H'' to L'', the same as H' to L' except that products of digestion are resorbed as soon as they are formed. Change indicated by figures under x lasted on the average 38 seconds; change shown by figures under y about $4\frac{1}{2}$ to 6 minutes.

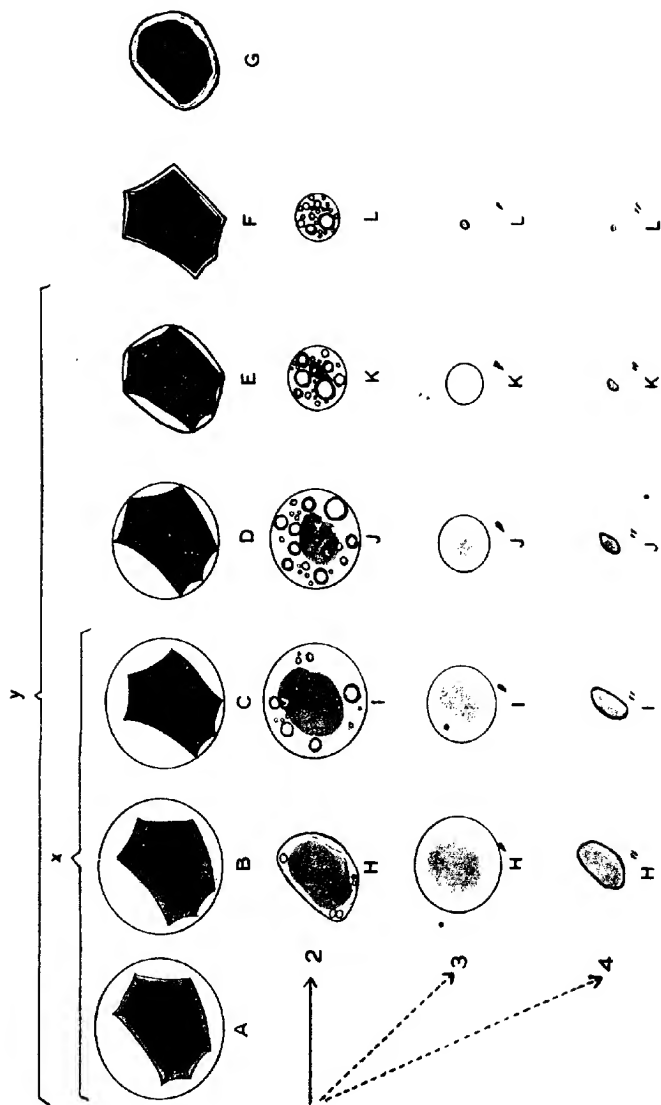
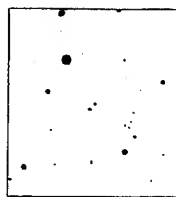
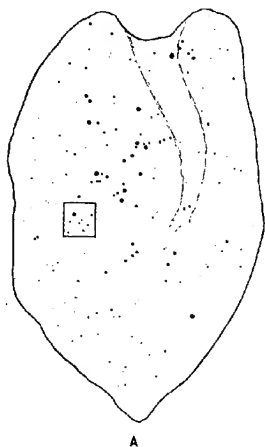


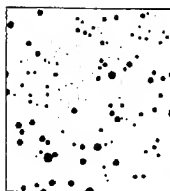
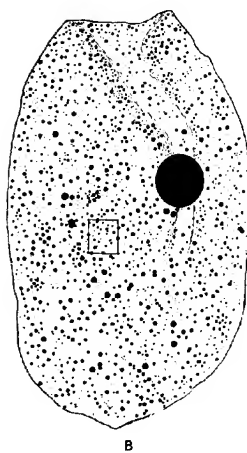
PLATE 2

EXPLANATION OF FIGURES

A and B Low power, camera lucida drawings of two sister cells stained to show fat 42 hours after separation. Cell A was not fed; cell B was fed olive oil 18 hours after separation from sister cell. The large drop of oil in *B* represents that remaining in the vacuole. *a* and *b*, high power, camera lucida drawings in one focal plane of area indicated by squares in *A* and *B*, respectively.



A



b

B

CHROMOSOME STUDIES IN THE DIPTERA

I. A PRELIMINARY SURVEY OF FIVE DIFFERENT TYPES OF CHROMOSOME GROUPS IN THE GENUS *DROSOPHILA*

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DIAGRAM AND TWENTY-SIX FIGURES (PLATE)

To the student of chromosomes and chromosome behavior a cytological study of the Diptera presents many features of interest. Some of these have been described or mentioned by Miss N. M. Stevens in connection with her work on the sex chromosomes,¹ but aside from this I know of no serious attempts at such a study. When compared with the detailed and critical works on the chromosomes of the Hemiptera, Orthoptera, and Coleoptera, this lack of knowledge of the Diptera is surprising, and can be explained only by the fact that the latter are in many ways unsatisfactory objects for cytological study. The difficulties in such a study are admittedly numerous, but notwithstanding, much can be accomplished, and the interest of the questions involved seems to me to more than justify the extra effort expended in their investigation.

For this reason a series of such studies has been undertaken, the extent of which will depend upon the time and facilities available. Some are under way at present and others will be taken up subsequently. The results I hope to present in a series of papers, to which the present is introductory.

¹ So far as known to the writer, the papers of Dr. Stevens are the only ones dealing with the chromosomes of the Diptera. They are four in number: The chromosomes of *Drosophila ampelophila*. 1907. Proc. VII Internat. Zool. Cong. A study of the germ cells of certain Diptera, with reference to the heterochromosomes and the phenomena of synapsis. 1908. Jour. Exp. Zool., vol. 5, pp. 359-374. The chromosomes in the germ cells of *Culex*. 1910. Jour. Exp. Zool., vol. 8, pp. 207-217. Further studies on the heterochromosomes of the mosquitoes. 1911. Biol. Bull., vol. 20, p. 109.

The object of this preliminary paper is to examine briefly the chromosomes of several species belonging to the genus *Drosophila*. This is a widely distributed genus and contains a large number of species, of which the most common is the ordinary 'pomace fly' or 'banana fly,' *Drosophila ampelophila*, already well known in genetic circles from the experimental work of Morgan and his students. Aside from *D. ampelophila*, about a dozen other species have been reared in the laboratory and studied cytologically. In the task of obtaining, breeding and identifying the flies I have been greatly assisted by Mr. A. H. Sturtevant, who has shown an active interest in the progress of the work from the beginning, and has kindly furnished cultures of several species which could not otherwise have been obtained. Some of the species studied have apparently never been described by taxonomists, and therefore, cannot be referred to by name in this paper. Consequently they are designated Species A, B, C, etc.

Among the twelve different species examined five distinct types of chromosome groups have been found,² each of which is characteristic of one or more species. These types, when compared with one another, chromosome by chromosome, show a relationship, which, if the hypothesis outlined below be true, may indicate an evolution of chromosomes in the genus. At all events, there is here presented a series of related chromosome groups more complete than has been found in any other genus of animals or plants known to me.

Owing to peculiarities of the male *Drosophila*, it has been necessary to take the data for this study principally from female specimens. The males, unlike those of most insects, are much less satisfactory for chromosome studies than the females, and therefore have been reserved for separate treatment. The male chromosome groups of two or three species are included, as mentioned in the text, but otherwise only the diploid female groups are considered. The latter are sufficient for present purposes, since only the normal somatic divisions are involved, and in these

² The chromosomes of one species, *D. ampelophila*, have been previously described by Dr. Stevens, ('07, '08).

it may be assumed that both sexes agree, save as regards the two sex-chromosomes (heterochromosomes).

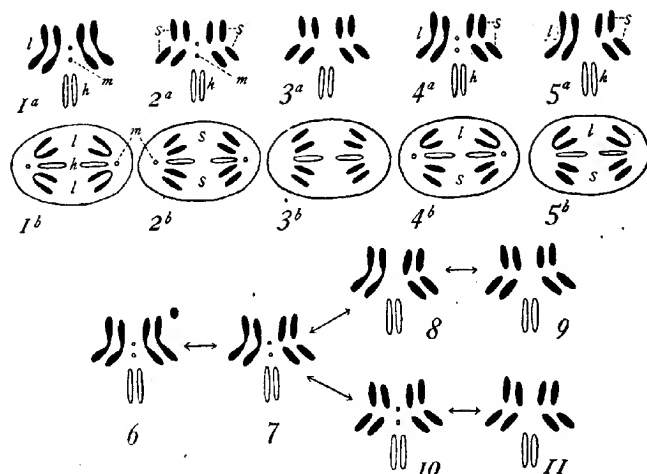
No attempt is made in this paper to elaborate the data. That can be done only after a more extensive study, and one involving more species. Likewise the figures have been limited to a number sufficient merely to illustrate the essential facts.

TYPE I

Chromosome groups found in Drosophila amoena, D. ampelophila, D. quinaria, and two species as yet unnamed

Figures 1 to 5 show metaphase plates of chromosome groups designated as Type I. Figure 1 is a diploid group of *D. amoena* female. Figures 2 and 3 are corresponding groups from males of the same species, introduced here for reference. In figures 4 and 5 similar groups are shown for *D. ampelophila* females. Figure 1 is from a section and indicates the normal relations of the chromosomes, except that one member of the small pair is hidden. The other four are from aceto-carminic smear preparations, and consequently show the chromosomes somewhat out of place or disarranged. Yet the essential similarity of all five figures is quite evident, and in each one the same constellation of chromosomes is readily seen. It is this constellation that is termed Type I.

If now, we examine any one of the figures, for instance figure 5, we immediately distinguish three different sorts of chromosomes on the basis of differences in size and form. The first sort is very small and spherical (fig. 5, *m*), the second elongate, rod-like or curved (fig. 5, *h*), and the third very long, more or less V-shaped and constricted in the middle (fig. 5, *l*). As a rule these chromosomes are definitely associated in pairs, there being in this group one pair of the first sort, one of the second, and two of the third—four pairs in all. The three classes, separated thus according to size and shape, are also distinguished by characteristic differences in behavior, which exhibit themselves in metaphase, anaphase and prophase. Upon this basis they may be named and characterized as follows:



Diagrams 1 to 5 Diagrams 1^a, 2^a, 3^a, 4^a, 5^a represent metaphase plates of Types I, II, III, IV and V respectively. Diagrams 1^b, 2^b, 3^b, 4^b, 5^b represent anaphases of the same. In these anaphases, for the sake of simplicity, only one member of each chromosome pair is figured; *m*., microchromosomes; *h*., sex- or heterochromosomes; *l*., large autosomes; *s*., small autosomes.

Diagrams 6 to 11 The same types of chromosome groups as above, but arranged in a dichotomous series according to their relationship to one another, and their divergence from Type I. 6, Type I; 7, Type IV; 8, Type V; 9 and 11, Type III; 10, Type II. As the diagrams indicate, Type III may be derived from either Type V or Type II.

1. The *m* or *microchromosomes*.³ One pair; small, spherical; usually occupying the center of the plate in metaphase; going to the poles as short, straight rods in anaphase (*m*, diagrams 1^a and 1^b).

2. The *sex-chromosomes* or *heterochromosomes*. One pair; unequal in the male,⁴ equal in the female; elongate, rod-like,

³ A name first applied by Wilson to a similar small pair seen in many coreid Hemiptera; (E. B. Wilson, Studies on chromosomes. II. 1905. Jour. Exp. Zool. vol. 2, pp. 508-545). Whether the pair in *Drosophila* is strictly comparable with these in the Hemiptera is not certain, and I have used the term here merely for convenience. It remains to be seen how the pair behaves in the maturation stages.

⁴ This inequality in the male was first pointed out by Miss Stevens ('08) in the case of *D. ampelophila*. In so far as the diploid chromosome groups of this species

usually lying radially in metaphase, i.e., one end pointing toward the center, the other toward the margin of the plate (diagram 1 a, *h*); attached to the spindle fiber at *one end*, and going to the poles as elongate straight rods, (*h*, diagram 1 b, and fig. 6); contracting precociously in prophase.

3. *The large autosomes.* Two pairs (in Type I); very long, more or less dumb-bell-shaped, with a definite constriction in the middle (*l*, diagram, 1 a and fig. 5); usually V- or U-shaped in metaphase, the apex of the *V* coinciding with the constriction and pointing toward the center of the plate, thus bringing the arms of the *V* into the position of radii pointing toward the margin; attached to the spindle fiber, not at one end, but at the *median, constricted part*, and going to the poles in V-shape (*l*, diagram 1 b and figs. 6 and 7).

The essential features in the behavior of these different sorts of chromosomes are indicated in figures 1 to 7 and diagrams 1 a and 1 b. Figure 7 shows an anaphase in polar view illustrating the difference between the V-shaped, large autosomes and the straight sex-chromosomes. Only one pole is figured, the other being almost identical with it. In this figure the *m*-chromosomes are not evident. Figure 6 shows the same features in side view. It is taken from the second maturation division of the male, involving only the haploid number of chromosomes (4), and although one pole is somewhat confused the figure shows the decided difference between the two kinds of chromosomes (*l* and *h*).

are concerned, my observations are entirely in accord with hers. But in the maturation divisions, where she describes a distinct *X* element separable from the *Y* of which it was formerly an integral part, I fail to corroborate her statements. So far as my observations go, they indicate an unequal *XX* pair in the male, without any additional piece attached to either. Neither my observations nor those of Miss Stevens are conclusive, however, owing to the difficulty of observing the chromosomes in these stages. The question is important for the bearing it has upon the breeding experiments with this fly, and we are doubly unfortunate in being thus far unable to settle it. By a recent examination of males of another species, having the same type of chromosomes as *ampelophila*, and showing more favorable conditions for their study, I have been led to hope that through this species some light can be thrown on *ampelophila*.

TYPE II

Chromosome groups found in Drosophila repleta, Species A and Species B

The type of chromosome group exhibited by these three species differs very decidedly, at first sight, from that just studied. It may be best understood by examining diagram 2 (a and b) and figures 8 to 13. The figures in this case are all but one (fig. 9) taken from fixed and sectioned material, and therefore show more nearly the proper arrangement of the chromosomes. Examining the individual chromosomes, as was done in Type I, two kinds found in that type are seen to be present here also. These are the small *m*-chromosomes and the sex-chromosomes (in this case the longest pair); as indicated in diagram 2. In addition to these two kinds there remain four pairs of short, rod-like autosomes in place of the two pairs of long, dumb-bell-shaped large autosomes of Type I. These constitute a fourth kind of chromosomes and may be characterized as follows:

4. The *small autosomes*. Short, rod-like autosomes without any median constriction; almost invariably straight in metaphase, and lying in a radial position, i.e., pointing from the center toward the margin of the plate (figs. 8, 10, 12); attached to the spindle fibers at one end, thus going to the poles as straight rods (s, diagram 2 a and 2 b).

The principal interest of Type II as compared with Type I centers around these eight small autosomes, which, it appears, replace the four large autosomes of Type I. This latter fact at once suggests the idea that each of the large autosomes has parted in the middle, at the point of constriction, and has thus given rise to two small ones. In this way the four large dumb-bell-shaped chromosomes could have been readily transformed into the eight small ones. The evidence of size, form, and behavior of the two kinds supports this view in a rather surprising manner. Diagram 2 (a and b) are designed to show this relationship schematically. They are purposely made very schematic, but the essential features are entirely substantiated by actual division figures. The

significant fact is that each of the small autosomes resembles in size, form and behavior one arm, or one end of a large autosome. So that by assuming a partition of the large autosomes at their median, constricted points, and assuming that each of the resulting halves behaved after separation just as it did before, exactly such a condition would be brought about as that found in Type II.

TYPE III

Chromosome groups found in Drosophila funebris

The important features in this type are shown in figures 14 to 17, and diagram 3 a and 3 b. In the main it agrees essentially with Type II except that the *m*-chromosome pair appears to be entirely lacking (compare figs. 8 and 14). Whether this disappearance has been brought about by the degeneration of the pair or by a fusion with some other pair I have been unable to determine. The unusual length of the sex-chromosomes might suggest the latter, but in view of the radical difference in behavior between them and the autosomes, and the fact that another type (V) also lacks the *m*-chromosomes, without a corresponding enlargement of the sex-chromosomes, I am inclined to doubt this explanation.

It is worthy of note that in this type (III) the sex-chromosomes are fully as elongate as the large autosomes of Type I, yet they retain their own characteristic form and behavior, and show no evidence of the V- or dumb-bell-shape, or a median attachment to the spindle fibers. If it were true that all chromosomes are qualitatively alike, and that form and behavior are merely secondary characteristics determined by size and bulk, then we should expect these long sex-chromosomes to assume the characteristics of the large autosomes of Type I, but as a matter of fact they do nothing of the sort. To my mind there is no clearer demonstration than this of a qualitative difference between the individual chromosomes of a group.

TYPES IV AND V

*Chromosome groups found respectively in Species C
and D. tripunctata*

Like Types II and III, these two differ from one another mainly in the presence and absence of the *m*-chromosomes. Otherwise they are essentially alike, and may be considered together. Figures 18 and 19 show early metaphases of Type IV taken from aceto-carminic smear preparations. Figure 20 shows the haploid number of the same, in the second spermatocyte division (equation division), from a section. In figures 21 to 26 are shown the diploid chromosomes of Type V, taken from sections. The first two figures represent metaphases—21 a polar view, and 22 a somewhat diagonal view. They differ in proportions from the figures of Type IV because of the contraction incident to fixing, embedding, etc., but nevertheless show their general similarity to the other type; (compare figs. 18 and 22). Figure 23, a late prophase of Type V, represents an earlier stage, before the autosomes have fully contracted. Figure 24 is an early prophase, included here to show the precocious contraction of the sex-chromosomes, and the very intimate pairing or conjugation of the autosomes. In figures 25 and 26 are shown the two poles of an anaphase, indicating the behavior of the three different kinds of chromosomes at this stage.

These two types (IV and V) are less easily analyzed than the previous ones, but the evidence clearly indicates that they are, in a way, intermediate between certain of the latter. Comparing Type IV with Type I, as indicated in diagrams 4 and I, it would seem that two of the large autosomes of Type I have undergone partition, giving rise to four small autosomes, while the other large pair has remained intact. By a similar partition of the latter a type like number II would be formed. From Type IV one like Type V could be derived by loss of the *m*-chromosome pair; as assumed in the case of Type III, either by degeneration, or by fusion with another pair. These various steps are indicated schematically in diagrams 6 to 11.

The principal difficulty encountered in the examination of Types IV and V, is that of distinguishing between the sex-chromosomes and the small autosomes. As shown by the figures, the likeness is so great that at certain stages they cannot be distinguished with certainty. This causes some trouble in late prophase and early metaphase, when the autosomes are in the process of contraction, but before and after this the separation may be made readily, so that the difficulty in no way interferes with the identification of the various chromosomes, or with the process of homologizing them with members of other types.

SUMMARY AND CONCLUSIONS

The genus *Drosophila* has numerous species which differ from one another in the number and form of their chromosomes, as well as in external characters. Of the twelve species examined by me five conform to one type in respect to the chromosomes, four conform to another type, and three to three additional types respectively—making five types in all. In each type the chromosome group is made up of certain different kinds of chromosomes, distinguished by their size, form and behavior. By comparing these types with one another an evolutionary series may be arranged. This series can be explained upon the assumption that four of the types arose from the first by a series of steps, involving, on the one hand, the partition of certain long chromosomes into halves, and on the other hand, the disappearance (by degeneration or fusion), in two cases, of a pair of very small *m*-chromosomes. It is not likely that the forms studied represent a consecutive series. In themselves they make a dichotomous series, but in all probability the intermediate steps in this constructed series, if they represent evolutionary stages at all, are merely indications of what has taken place, and do not themselves belong in the positions assigned to them.

Type I is arbitrarily chosen as the stem from which the others are derived because it includes the greatest range of species, and because it is easier to conceive of an evolution by partition, or by loss, of chromosomes than by their fusion or generation. The

direction of evolution, however, is purely a matter of opinion and may quite readily be reversed.

As far as the characters of the insects themselves are concerned, they throw little light on the question. The various species are, for the most part, quite different from one another, with the exception of two, and these have similar chromosomes (Type I). From the taxonomic standpoint the genus has never received adequate attention from entomologists, and consequently there is little clue as to the relationship of species.

DISCUSSION

A detailed discussion of the various questions suggested by this study would be out of place at this time, but it may be profitable to call attention briefly to some phenomena appearing in these flies, which do not present themselves so conspicuously in other animals.

Individuality of the chromosomes

In practically all the species used in this study the metaphase plates stand out as clearly as diagrams, and in most cases there cannot be the slightest doubt as to the identity of any particular kind of chromosomes. Especially is this true of such types as II and III, in which most of the chromosomes are short. And even in the other types where more long chromosomes are present there is no difficulty in identifying the different sorts except at certain stages. Consequently, after studying scores of preparations from larvae, pupae and adults, and finding that in each of these the same particular kinds of chromosomes appear over and over again with the greatest regularity, both in the somatic and germinal tissues; and then after noting that each of these kinds has its own particular method of behavior in each cell division, I can find little reason to doubt that every chromosome has a specific character of its own, and that its individuality persists from one generation to another. Upon no other assumption can I explain the exact repetition of identical chromosome groups in certain species, and the appearance in other species of groups so related to one

another and to the first that the individual chromosomes can be homologized throughout the entire series, as they can be in the *Drosophilas*.

Pairing and conjugation of chromosomes

The *Drosophilas* offer some of the most striking evidences of the actual pairing of chromosomes in the diploid groups, thus far observed. The phenomenon is apparently characteristic of all Diptera,⁵ but is nowhere so striking as in this genus.

The figures speak for themselves in this regard, and need little emphasis. The sex-chromosomes alone show any appreciable number of exceptions to such a paired association. A number of cases have been found in which these were not paired, and in general the association between them is less intimate than that between members of autosome pairs. Whether this is correlated with the precocious contraction of the sex-chromosomes, or is due to an inherent difference between them and the autosomes, cannot be determined. In any event, the difference is slight, for normally they are paired like the autosomes.

In such cases as these, where two or three pairs of chromosomes can be individually identified, there can be no question that *each pair is composed of one maternal and one paternal component*, since any germ cell of either sex contains only one member of each of these kinds. And when it is evident that three out of five pairs are so made up (e.g., Type IV), a very strong presumption is created that the other two pairs are similarly constituted.

But the most remarkable feature of the whole study is the discovery that the chromosomes not only exhibit a close association in pairs at nearly all times, but that before every cell division the members of each pair become so intimately united that they may be said actually to conjugate. Each pair, with the possible exception of the sex-chromosomes, goes through what amounts to •a *synapsis* in every cell generation, so that in many cases the

⁵ Miss Stevens ('08, '11) records it in nine species of Muscidae, and four species of mosquitoes. I have verified it in five of these species of Muscidae, and extended it to eight others, in addition to those of *Drosophila*.

figures closely resemble those of haploid groups. Apparently this takes place especially in early prophase (fig. 24), but a second conjugation may occur during metaphase, just a short time before division (figs. 9, 15, 16). Whether in the first case conjugation takes place in anaphase and continues into the succeeding prophase has not been ascertained. This interesting question is being given special study at the present time, with a view to determining the exact nature and extent of the conjugation. In the second, or metaphase conjugation, at least, it is worthy of note that the union is unquestionably a side-by-side, or *parasynaptic* one.

It need hardly be urged, in the face of such evidence as this, that an actual pairing of chromosomes may take place in the diploid nuclei outside of the sphere of reduction. True, there is little evidence, in most organisms, of such a side-by-side association of chromosomes to form pairs. But the fact that it occurs so strikingly in some forms adds great weight to the conclusion (based on size relations and other evidence), that such pairs are present in all, even when the members are not obviously associated. If anyone still doubts the reality of pairing of homologous chromosomes, I should recommend to him a study of the Diptera.

In conclusion, I wish to express my appreciation of the kindly assistance and advice of Professors E. B. Wilson and T. H. Morgan of this laboratory. And I wish to thank Dr. C. B. Davenport for the many courtesies extended to me during my stay at Cold Spring Harbor.

Addendum. Since this paper went to the publisher, further study of *D. funebris* (Type III) has revealed the presence of a minute pair of *m*-chromosomes in at least some specimens of this species. Apparently either the size of the *m*-chromosomes is variable in *D. funebris*, or else these chromosomes are absent in some specimens and present in others.

PLATE

PLATE I

EXPLANATION OF FIGURES

All figures, with the exception of figure 9, were drawn with the aid of a camera lucida, using Zeiss 1.5 mm. apochromatic objective, and compensating ocular 12, with tube length of 160 mm. The drawings are reproduced natural size. Figure 9 was drawn freehand, but to about the same scale as the others. For the preparation of figures 6, 9, 17, 23 and 24 I am greatly indebted to Miss Mabel L. Hedge. Figures designated as 'oogonial' may be, in some cases, from ovarian follicle cells; the two cannot always be distinguished.

1-7 Chromosome groups of Type I.

1 *Drosophila amoena*, oogonial metaphase; from a section (one *m*-chromosome concealed).

2 and 3 *D. amoena*; spermatogonial metaphases; aceto-carmin.

4 and 5 *D. ampelophila*; oogonial metaphases; aceto-carmin.

6 *D. amoena*; second spermatocyte anaphase; side view; showing haploid number of chromosomes; section.

7 *D. quinaria*; one pole of anaphase; oogonial division. Polar view showing contrast between large autosomes and sex-chromosomes; *m*-chromosomes not shown; section.

8 to 13 Groups of Type II.

8 *D. repleta*; oogonial metaphase; section.

9 *D. repleta*; oogonial metaphase slightly earlier than figure 8, showing conjugation of chromosomes; aceto-carmin.

10 *D. (Species A)*; oogonial metaphase; section.

11 *D. (Species B)*; early metaphase or late prophase; section.

12 and 13 Same; later stage; sections.

14 to 17 Type III.

14 and 15 *D. funebris*; metaphase from ovarian cells; sections.

16 *D. funebris*; earlier than last, showing conjugation of autosomes; section.

17 *D. funebris*; anaphase, polar view showing both poles; All chromosomes rod-like; the autosomes completely divided and daughter halves separated; the sex-chromosomes just pulling apart, but still attached at one end; section.

18 to 20 Type IV.

18 and 19 *D. (Species C)*; oogonial metaphase; aceto-carmin.

20 Same; second spermatocyte metaphase; chromosomes partly divided; section.

21 to 26 Type V.

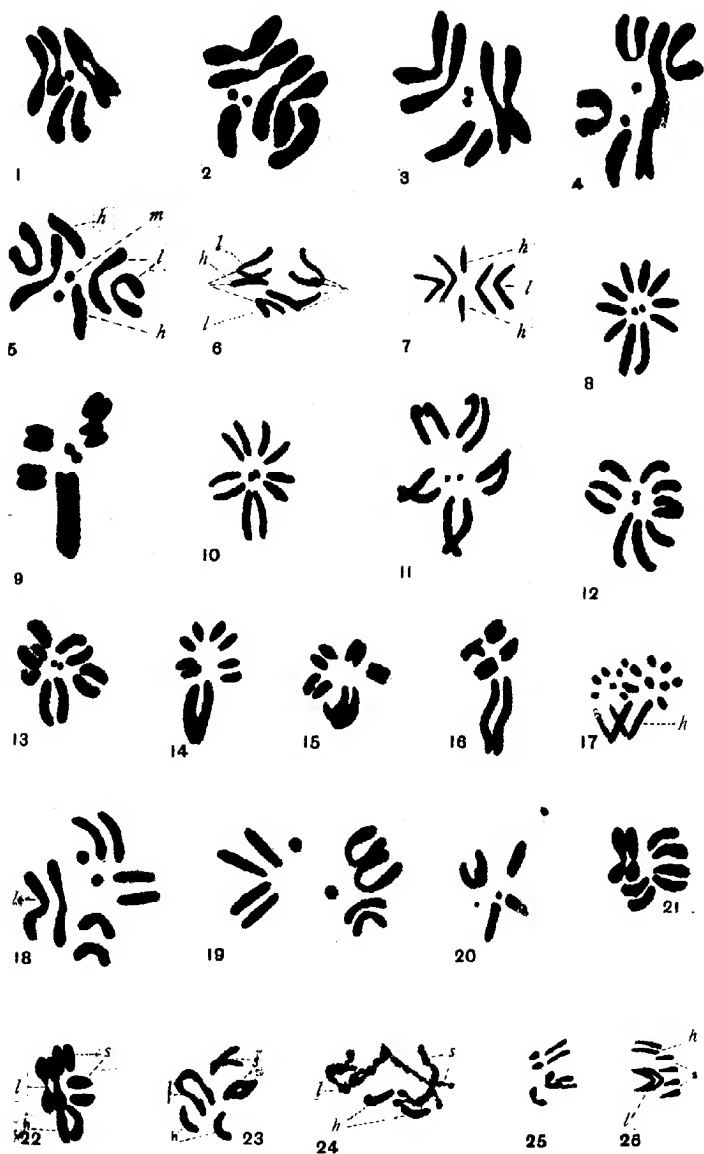
21 *D. tripunctata*; polar view of metaphase from ovarian cell; section.

22 *D. tripunctata*; diagonal view of metaphase; slightly later stage; section.

23 *D. tripunctata*; late prophase from ovarian cell; section; *h*, sex-chromosomes.

24 *D. tripunctata*; early prophase from ovarian cell; autosomes conjugated in pairs; section.

25 and 26 *D. tripunctata*; two poles of anaphase from ovarian cell. Show V-shaped large autosomes (*l*), rod-like small autosomes (*s*), and rod-like sex-chromosomes (*h*), section.



STUDIES ON THE DYNAMICS OF MORPHOGENESIS AND INHERITANCE IN EXPERIMENTAL REPRODUCTION

VIII. DYNAMIC FACTORS IN HEAD-DETERMINATION IN PLANARIA

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TWO FIGURES

In earlier papers (Child '11 b, '11 c, '11 e) attention has been called to the fact that the frequency of head-formation in isolated pieces of *Planaria dorotocephala* varies with size of piece, region of body and various external conditions which can be controlled experimentally. This difference in capacity, together with the possibility of comparing rates of metabolism in different pieces by the susceptibility method (Child '13 a), affords a means of determining why some pieces produce a head and others do not and since the formation of the head is the first step in the development of the new individual we have an answer to the question why some pieces give rise to new wholes and others do not.

I. THE TIME OF HEAD-DETERMINATION

Since it can be determined experimentally within wide limits in various ways whether a piece of *Planaria dorotocephala* shall give rise to a head or not (Child '11b), it is evident that the fate of the piece as regards head formation is not fixedly and finally determined at the time of isolation of the piece from the parent body. The first step in the analysis of the factors concerned in head determination is to find when the determination of the head occurs. This can be readily and very simply done by the following method.

Short pieces from the middle region of the body, i.e., the posterior region of the first zoöid of *Planaria dorotocephala*, such for example, as *ab*, *bc* and *cd* in figure 1, give rise in 98 to 100 per cent of the cases, under ordinary conditions, to headless forms (Child

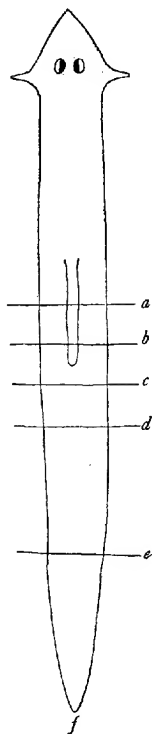


Figure 1

'11 b, '11 c), i.e., no outgrowth of new tissue occurs at the anterior and beyond the healing of the wound.

On the other hand, long pieces with anterior ends at the same levels as those of the short pieces (pieces like *ae*, *af*, *ce*, *cf*) produce under the same external conditions 98 to 100 per cent of animals with fully developed normal heads.

It is of course evident that in these cases the length of the piece is in some way a factor in head-formation, but the question how it affects head-formation will be answered later. At present we are concerned merely with the fact that the cells at the levels, *a*, *b*, etc., when at the anterior ends of short pieces, do not give rise to heads, but when at the anterior ends of long pieces do give rise to heads. In order to find approximately when head-formation is determined, it is only necessary to prepare a large series of the long pieces, such as *af* or *cf* in figure 1, and at different intervals after section to remove short pieces such as *ab* or *cd* from the anterior ends of a certain number. We know that such short pieces when completely isolated at once do not develop heads. If heads appear at their anterior ends after they have remained a certain length of time as the anterior regions of long pieces and have then been isolated, it is evident that the formation of heads must have been determined while they were still a part of the long piece. The records of experiments will show the character of results obtained.

Series 458. From well fed worms 18 mm. long, about 150 pieces, including the region *cf* in figure 1, were prepared and from these the following five lots were taken.

Lot I. From the anterior ends of twenty-five of the long pieces, short pieces like *cd* were removed 15 to 30 minutes after the long pieces were prepared.

Lot II. From another twenty-five of the long pieces, similar short pieces were cut 3 to 4 hours after the long pieces were prepared.

Lot III. Twenty-five similar short pieces 7 to 8 hours after first operation.

Lot IV. Twenty-five short pieces 18 hours after first operation.

Lot V. Twenty-five short pieces 24 hours after first operation.

These five lots of short pieces were allowed to undergo reconstitution at a temperature of 20 to 22°C. and when this was complete the character of each piece was recorded; the results appear in table 1.

The five types distinguished have been described in earlier papers (Child '11 b, '11 c). The normal form possesses a head

with two eyes and two lateral cephalic lobes, as in figure 1. In the teratophthalmic form the eyes are either partially or wholly united or unequal in size or abnormally placed. The teratomorphic form has a single eye in the median line and the cephalic lobes appear on the front of the head and may be partially or completely united in the median line. In the anophthalmic form an anterior outgrowth is present, containing a small abnormal ganglionic mass, but eyes do not appear. And finally, the headless form shows no anterior outgrowth except closure of the wound.

The increase in head-frequency in the short pieces is evident from table 1. In Lot I, 80 per cent remain headless and only 8 per cent give rise to heads with eyes of any kind and none are

TABLE 1

LOTS	TIME BET. 1ST AND 2D OPERA- TION IN HRS. AND MIN.	NORMAL	TERATOPH- THALMIC	TERATO- MORPHIC	ANOPH- THALMIC	HEADLESS	DEAD
I	0.15-0.30		4	4	12	80	
II	3.00-4.00	4	32	4	24	28	8
III	7.00-8.00	28	60			12	
IV	18.00	76	16			4	4
V	24.00	80	8				12

normal. But Lot II shows that after 3 to 4 hours the anterior ends of the long pieces have been determined as heads to such an extent that their isolation as parts of short pieces prevents head-formation completely in only 28 per cent, while 24 per cent are anophthalmic and the rest form heads with eyes, 4 per cent normal, 32 per cent teratophthalmic and 4 per cent teratomorphic. In Lot III, where the short pieces were cut off after 7 to 8 hours as parts of the long pieces, only 12 per cent remain headless and 28 per cent are normal and 60 per cent teratophthalmic. In Lot IV, after eighteen hours, 76 per cent are normal and 16 teratophthalmic, and in Lot V, after twenty-four hours, 80 per cent are normal. After 18 hours the head is so fixedly determined in practically all cases that isolation of the head-forming region as part of a short piece cannot prevent head-formation. The approach in

the character of the head to the normal, as well as the increase in head-frequency, is also evident. In Lot II only 4 per cent of normal heads appears, but in Lot III 28 per cent are normal, in Lot IV, 76 per cent and in Lot V, 80 per cent. This series shows that head determination begins almost at once after section and that in most pieces, the head is fixedly determined within seven to eight hours after section (Lot III, 88 per cent form heads).

The following series gives in general the same results but brings out some points more clearly than the preceding.

TABLE 2

LOTS	TIME BET. 1ST AND 2D OPERA- TION IN HRS. AND MIN.	NORMAL	TERATOPH- THALMIC	TERATO- MORPHIC	ANOPH- THALMIC	HEADLESS	DEAD
Controls		72	28				
I	0					96	4
II	2.30-3.00	4	16		12	68	
III	5.30-6.00	4	28	8	24	32	4
IV	12.00	36	60			4	

Series 465. From well-fed worms 15 to 16 mm. in length, about 150 long pieces like *ae* (fig. 1) were cut.

Lot I. Twenty-five short pieces (*ab*, fig. 1) cut immediately after first operation.

Lot II. Twenty-five short pieces cut $2\frac{1}{2}$ to 3 hours after first operation.

Lot III. Twenty-five short pieces cut $5\frac{1}{2}$ to 6 hours after first operation.

Lot IV. Twenty-five short pieces cut 12 hours after first operation.

All lots kept at 20 to 22°C. The results appear in table 2, in percentages.

The controls, i.e., twenty-five of the long pieces, all form heads, 72 per cent normal and 28 per cent teratophthalmic. On the other hand, Lot I, consisting of twenty-five of the short pieces removed at once from the anterior end of the long piece, develops no heads at all, 96 per cent being headless and 4 per cent dead.

The contrast in head-frequency between the long and short pieces is strikingly shown in these two lots. It must be remembered that in the two cases the level of the body concerned in head-formation is the same, or as nearly as possible the same, yet in the long pieces 100 per cent of heads are formed and in the short pieces none. It may be added further that in pieces of intermediate length all possible intermediate percentages of head-frequency are found.

Comparison of Lots I to IV of the short pieces shows essentially the same result as table 1 above. In Lot I all remain headless, in Lot II, 68 per cent, in Lot III, 32 per cent and in Lot IV, 4 per cent. The table also shows that not only does the head-frequency increase but that a marked approach to the normal in the character of the head occurs, as the length of time during which the short piece remains as an anterior portion of the long piece increases. In Lot II, 20 per cent of the pieces form heads with eyes, in Lot III, 40 per cent, and in Lot IV, 96 per cent.

The only possible conclusion from these two series is that the factors which determine whether an isolated piece shall give rise to a head or not, begin to act almost immediately after the operation; that within 3 hours after section, the determination between 'head' and 'headless' has already occurred in about 50 per cent of a lot of pieces under the usual conditions and that this determination has occurred in practically 100 per cent within twelve hours after section.

It also appears from the two tables that the determination of the character of the head formed occurs somewhat later than the determination whether a head shall be formed or not. At 3 hours and even at 6 hours, anophthalmic as well as normal and teratophthalmic forms appear in considerable percentages (table 2) and even at 12 hours the percentages of normal heads is only half that in the controls. At 18 hours, however, most heads are determined as normal (Lot IV, table 1).

These and other similar series in which worms of approximately the same size and physiological condition were used and in which external conditions are as nearly as possible uniform, all give similar results. Under these conditions, head-formation

is fixedly determined in most pieces within 8 hours after section and in practically all, within 12 hours after section. The determination of the character of the head occurs later. In this way it is possible to discover approximately the time of determination, not only of the head as a whole, but of the cephalic lobes, eyes and preocular region and of the various types of head. For comparable results animals of similar size and condition and similar external conditions are necessary, because the time of head-determination varies both with internal and external factors and in fact can be altered experimentally.

The times obtained in this way, however, represent the times when the structures concerned have become so fixedly determined that they cannot be altered even by extreme changes in conditions. There is every reason to believe that in a given piece it is determined whether a head shall form or not some time before that determination becomes so firmly fixed as to be unchangeable by altered conditions. Tables 1 and 2 show that in a considerable percentage of pieces, head-determination has become fixed within 3 hours after section. In short, there is no question that under the usual conditions head-determination in pieces must occur, or at least begin, almost immediately after section and the conditions existing in the piece during the first two or three hours after section must constitute the most important factors in the process of head-determination.

II. HEAD-FREQUENCY AND DEGREE OF STIMULATION IN PIECES

In the first paper of this series (Child '11 c) it was shown that the frequency of head-formation in pieces under the usual conditions of temperature, etc., decreases with decrease in length and also with increasingly posterior level of the piece within the limits of a single zoöid (Child '11 e). In other words, the shorter or the more posterior a piece is, the less likely it is to give rise to a head. In the preceding paper (Child '14 b) it was found that the amount of temporary increase in the rate of metabolism, i.e., of stimulation of pieces resulting from section, increases with decreasing length and increasingly posterior level of the piece within the limits of a single zoöid.

Bringing these two groups of facts together, we see that *frequency of head-formation decreases as stimulation following section increases*. This relation seems at first glance somewhat paradoxical, for it means essentially that the lower the rate of metabolism in a piece in general, the more likely it is to give rise to a head, and vice versa, but in this and following papers evidence will be presented to show that this relation holds within certain limits.

It was demonstrated in the preceding section that head-determination occurs within a few hours after section, at the latest, in other words, during the period of stimulation following section, which lasts for several hours and is followed by a gradual fall in rate of metabolism. We are then forced to the conclusion that a relatively high rate of metabolism in the piece as a whole, acts in some way as a factor inhibiting head-formation. This conclusion appears somewhat revolutionary, for it is generally believed that the development of a new head on a headless piece is a process of replacement of a missing part and that it is determined and controlled by other parts of the piece. But if the facts cited above are correct, the more vigorous the piece and the more capable it is of determining and controlling processes in other parts during the period when head-determination occurs, the less frequently does a head arise from it. This can mean only that the maintenance of the piece and the process of head-formation are in some way opposed or antagonistic to each other, and that the new head is not determined by the piece, but rather in spite of it. This point of view has already been briefly stated, together with some of the evidence on which it is based (Child '13 b), but further consideration is necessary to make clear its important features and its significance for our conception of the individual and its development.

III. THE PROCESS OF HEAD-DETERMINATION

The head arises from cells adjoining the anterior cut surface of the piece, which are so greatly affected by the altered correlative conditions and the presence of the wound that they lose their differentiation more or less completely, begin to divide rapidly and produce the outgrowth of new tissue. From this

mass of new embryonic tissue the new head arises. This head then develops from the earliest stages of morphogenesis, while in other regions of the piece, except at the posterior end, the original structure undergoes more or less alteration but does not completely disappear in a regression to embryonic cells. In figure 2 the region of head-formation is indicated by the shaded region at x , the region of tail-formation by z , and those regions of the piece which retain the chief features of their structure, by y .

We have already seen that the higher the rate of metabolism in y following section, the less likely is a head to arise from x , and vice versa, and it has been pointed out that this inverse re-



Figure 2

lation between head-frequency and rate of metabolism in other regions of the piece, can mean only that the new head develops, not in correlation with and under the control of the piece as a whole, but, so to speak, in spite of it.

In short, the facts already cited, and a large body of evidence still to be presented, point directly to the conclusion that head-formation in a headless piece is not the restitution of a missing part but the first step in the development of a new individual. If this is the case, then head-formation in a piece is essentially the same process as in embryonic development. There the head, or more specifically the cephalic nervous system, is the first region or organ of the individual to become morphologically visible. So far as we can determine, these early stages of head-development are not dependent upon conditions in other regions of the egg or embryo, but the head-region takes the lead in development.

In an earlier paper (Child '12) the writer has shown that in Planaria reconstititional development may be inhibited in various degrees by low concentrations of narcotics and that the formation of a head may still occur when all development of other parts is completely inhibited. Moreover, it is a familiar fact that very short pieces of Planaria may give rise to a single head or to biaxial heads without other parts, and the same is true for Tubularia and various other forms. These various facts demonstrate that head-formation in Planaria is possible without any correlative influence of other parts, i.e., it is a process of 'self-differentiation' (Child '13 b, pp. 614-617). On the other hand, there is no evidence favoring the opposite conclusion. Even as regards pieces undergoing reconstitution, there are no facts indicating that head-formation is determined by other regions of the piece. We have simply been accustomed to consider that the piece replaces lost parts and so gives rise again to a new whole. But it has often been pointed out that in such forms as Planaria, the piece does not replace exactly the parts lost, but a new whole arises by the formation of a head at one end, a posterior end at the other and the reorganization of remaining portions. What actually occurs in these cases is that a new head region begins to develop as the first step in a new individuation and this new head region dominates other parts and determines a reorganization of the old tissues of the piece. Head-formation is not determined by other parts, but it—or more specifically the formation of the cephalic nervous system—represents the fundamental morphogenetic reaction of the specific cellular material.

The existence in Planaria of the inverse relation between head-frequency and rate of metabolism in the piece as a whole, constitutes further important evidence in support of this view and is readily understood from this standpoint. It can mean only that the cells of the region x (fig. 2) give rise to a head except in case the rate of metabolism in the region y is sufficiently high to retard or inhibit this process of "self-differentiation."

The rate of metabolism in the cells at x , which we may call 'rate x ,' is probably determined largely by local conditions connected with the altered correlative conditions and the presence

of the wound. Under given external conditions, it probably does not differ very greatly in pieces of different length and from different regions. The rate in the region y (rate y), on the other hand, varies, as we have seen, with size and level of body. When rate y is sufficiently high in relation to rate x , the cells at x are prevented from beginning independent development and producing a new head. On the other hand, the lower the rate of y in relation to the rate of x , the more independent are the cells at x and the more likely to produce a head.

The fact that head-determination in pieces occurs almost immediately after section, shows that the critical period is at the very beginning of the division and growth in the region x . Evidently, the determination whether a head shall arise or not is essentially simply a question whether the region x shall develop with at least a certain degree of independence of the region y , in which case it produces a head, or whether its development shall be inhibited by y .

If this conception of the process of head-determination is correct, then we arrive at a very simple expression for the head-frequency in pieces of different size and from different regions, viz., head-frequency = $\frac{\text{rate } x}{\text{rate } y}$.

We do not know positively whether rate y must actually be higher than rate x in order to inhibit head-formation, but there is no reason to believe that such a difference is necessary. It has been pointed out in earlier papers (Child '11 d, '12, '13 c) that in the intact animal the rate of metabolism decreases from the head region posteriorly. If this is the case and if the effects of section and the local effect of the wound could be eliminated, rate x in a piece should always be higher than rate y . But the region y is a system of correlated parts with conducting paths and metabolic mechanisms fully developed and capable of a relatively high degree of stimulation. The region x , on the other hand, during the first few hours after section (i.e., the period when its fate is determined) is merely a group of cells without definite mechanism of correlation corresponding to a head. It is possible that the fully developed region y may overbalance and inhibit

the few cells at x , even though rate y per unit of weight or volume is not actually higher than rate x . The development of conducting paths, and in general the differentiation of y , must render it more capable of producing correlative effects upon other parts than are the few cells which are undergoing dedifferentiation at x . Probably the rate of metabolism, cell for cell, is higher in x than in y or becomes so very soon after section, for the cells at x are those most affected by section. Susceptibility experiments indicate that the region x usually possesses the highest rate of any part of the piece. But in order to be able to develop in spite of the region y , rate x must in all probability be considerably higher than rate y , and the differences in susceptibility of the regions x and y indicate that this is actually the case. In fact, before the new tissue has developed far enough to permit the distinction between heads and headless forms, we find in general a greater susceptibility in the region x as compared with y , in those pieces which would later show the higher head-frequency.

But whatever particular relation between rate x and rate y which may prove to be necessary for the inhibition of head-formation or the development of a head, the expression head-frequency = $\frac{\text{rate } x}{\text{rate } y}$ still serves to indicate the internal conditions which influence head-formation. And not merely head-frequency in general but the frequency of any of the different types of head, normal, teratophthalmic, teratomorphic, anophthalmic, is determined in the same way. It will be shown that all these different types of head represent simply different degrees of retardation of the process of head-formation, whether by external or internal factors. In very short pieces, where the whole or nearly the whole piece represents region x and region y is absent or very small, head-frequency should become proportional to rate x , and this is actually the case. Any conditions which decrease the rate sufficiently decrease the frequency of head-formation. This point will be considered more fully at another time. If the region x once succeeds in beginning its independent course of development it soon becomes the dominant region of the piece (Child '11 d), develops into a head and determines the establish-

ment of a new axial gradient and so the reorganization of the region y .

As regards the region z of figure 2 from which the posterior end develops, the relations to other parts are not as clear as in the case of x , for the chief visible differences in tail-formation are merely differences in amount of growth. In general, however, it is evident that development of the posterior end is directly proportional to rate y . But the stimulation following section has no relation to tail formation, because it is merely temporary and the tail is a subordinate part, depending for its very existence upon correlations with more anterior regions. It can form at any time, whenever rate y is high enough to determine its development. Tail-formation is retarded or inhibited by all depressing conditions, such as low temperature, low concentrations of narcotics (Child '12), etc., but exactly the same conditions may increase head-frequency in the same pieces. The experimental data upon this point will be presented at another time.

IV. THE FACTORS WHICH DETERMINE LOCALIZATION OF THE NEW HEAD

In all longer pieces of *Planaria*, the head when it forms is localized at the anterior end of the piece. The basis for this localization is the axial gradient: how the gradient determines the localization we have now to consider. Attention has already been called to the fact that when a planarian (earthworm, etc.) is cut in two, the posterior piece is much more strongly stimulated than the anterior. The same relation is evident in contact stimulation. Slight stimulation of a given region produces much more marked effects posterior than anterior to it. In short, the whole mechanism of dynamic correlation in *Planaria* and similar forms is developed on the basis of the axial gradient. Correlation between regions of the body is chiefly in the posterior direction, anterior regions being relatively independent of posterior, and posterior regions relatively dependent upon anterior. That metabolic gradients exist in at least some nerves in the lower animals is known (Child '14 a) and there are reasons for be-

lieving that conduction occurs more readily and to greater distances in the downward direction of the metabolic gradient. Conduction against the gradient is possible but requires a much stronger stimulus for a given distance. If the nerve impulse is a wave of chemical reaction, as seems now to be demonstrated (Tashiro '13), it is at least probable that in order to travel for any appreciable distance up the original gradient, the impulse must be strong enough to reverse the gradient temporarily as far as it proceeds, while a much weaker impulse may travel down the gradient for long distances. But whether this interpretation is correct or not, the fact remains that in planarians and many other lower forms, nervous and dynamic correlation (Child '11 a, p. 18) in general, is chiefly from more anterior to more posterior regions.

Admitting this fact, it follows that cells at the anterior end of a piece are in general more independent of other regions of the piece than the cells of any other level. It has been pointed out that the conditions necessary for head-formation are: first, embryonic cells of the species, second, physiological or physical isolation from correlative factors, and third, a sufficiently high rate of metabolism. It is evident that the condition of isolation from correlative factors is more completely fulfilled at the anterior end of the piece than elsewhere. At the posterior end, on the other hand, the new cells can never become physiologically isolated unless the axial gradient is eliminated, and in such cases we find that a head may actually arise at the posterior end of a piece. And in cases where the gradient is more or less permanently reversed, a tail may arise at the anterior end.

Briefly stated, the localization of new head and tail on a piece are due to the existence of the axial gradient. The head, which develops independently of other parts, develops at the anterior end because this is the only region of the piece where a sufficient degree of physiological isolation can possibly occur, as long as the axial gradient persists. The tail, which arises only as a subordinate part dependent upon and determined by more anterior regions, is localized at the posterior end of the piece because the cells in this region cannot become physiologically

isolated as long as the gradient persists, but develop under the dominance of more anterior parts and therefore produce a tail. By eliminating or reversing the gradient we may alter these localizations.

The headless piece represents a condition intermediate between the development of a head and of a tail at the anterior end. When rate y is sufficiently high in relation to rate x , to decrease the original gradient below a certain minimum in the anterior region of the piece, head-formation is simply inhibited, because neither x nor y dominates completely. But if rate y should become sufficiently high, as compared with rate x , to eliminate the old gradient and establish a new one sufficiently steep in the opposite direction in the anterior region of the piece, the region x will become a tail instead of a head. These points will be further discussed at another time on the basis of further experimental evidence.

V. THE FUNDAMENTAL REACTION SYSTEM OF THE SPECIES

Attention has already been briefly directed in other papers (Child '13 b, '13 c) to certain consequences for the problems of inheritance and development of the conception of the organism developed in these studies. If the head-region is a 'self-differentiating' system which arises in development independently of other parts and if other parts arise only in correlation with a head region or with a part which has already arisen in this way, we are forced to the conclusion that a single fundamental reaction system is the basis of both development and inheritance. The apical region or head region, or in animals which develop a morphologically differentiated nervous system, the cephalic region of the nervous system which is the dominant part of the head, is a closer approach than any other part of the organism to a morphological expression of this fundamental reaction system. In the lower animals, as well as in the plants, we see as a matter of fact that an isolated cell or group of cells capable of development produces an apical region or head except where it is prevented from doing so by correlation with already existing apical regions or heads.

The conclusion that the organism consists fundamentally of a single reaction system which is most closely represented morphologically by the apical region or head, or its dominant part, is forced upon us by the facts. Moreover, it is the only conception which enables us to account satisfactorily for the definite, orderly character of development, its progression in general from anterior to posterior regions, the dominance of the growing tip in plants and of the head in animals, and various other morphological and physiological characteristics of organisms. On the other hand, there are no real facts which indicate that the organism consists fundamentally of a multitude of independent determinants, factors or entities of any sort. Even the Mendelian phenomena do not demonstrate the existence of independent factors as entities, but merely indicate the existence of different capacities in a system, or of a variety of different systems. The localization of the visible realization of a capacity implies nothing as to the localization of the capacity. Any corpuscular theory of heredity and development demands the assumption of an anthropomorphic mechanism or 'vitalistic' principle of some sort for the management of the corpuscles. The conception of a fundamental reaction system as the basis of inheritance and development avoids all these as well as other difficulties; is developed from experimental data and brings into line a great number of facts which are very generally ignored by current theory.

The fundamental reaction system, dominance of the apical region and the axial gradient are all merely different aspects of the same general idea, viz., that the specific protoplasm of any organism consists fundamentally of a single physico-chemical reaction system which we may, if we desire, conceive as made up of a larger or smaller number of fundamentally similar partial systems. This system is the basis of inheritance and its dynamic capacities, the foundation of hereditary characters. The first step in organization and in embryonic development results from the establishment, in one way or another, of some region or portion of this protoplasmic reaction system as a region of higher

rate of dynamic activity. This region dominates development, becomes the apical or head region and determines the axial gradient or gradients, which constitute the dynamic basis of polarity and of individuation. The organization and development of various parts of the organism rests upon a similar basis of fundamental reaction system and dominance and subordination of parts resulting from differences in rate of reaction. Following papers will be devoted primarily to the development of this general conception on an experimental basis and its application to particular aspects of the problem of experimental reproduction, and secondarily, to the question of its significance for inheritance and development in general.

VI. SUMMARY

1. Whether or not a head shall arise at the anterior end of an isolated piece of *Planaria dorotocephala* is determined so fixedly during the first six or eight hours after section that head-formation cannot afterward be prevented by conditions which do prevent it when acting immediately after section. Head-determination undoubtedly begins immediately after section.

2. The period during which head-determination occurs is the period of stimulation following section, and in general the more a piece is stimulated by section the less likely it is to produce a head. Head-formation is a process opposed or antagonistic to the maintenance of the piece.

3. The development of a new head on a headless piece is not the restitution of a missing part but the first step in the development of a new individual. Whether a head shall develop or not depends primarily on whether the cells which give rise to new tissue at the anterior end of the piece become physiologically isolated to a sufficient degree to develop independently of other parts of the piece, or whether other parts prevent this development. In the former case a head arises, in the latter the piece remains headless.

4. If the group of cells which gives rise to the embryonic tissue at the anterior end of the piece is designated x , the similar group at the posterior end, z , and the remainder of the piece, y , we may express head-frequency in pieces in a very simple form, viz.: head-frequency = $\frac{\text{rate } x}{\text{rate } y}$. Tail-frequency, on the other hand, is directly proportional to rate y .

5. In pieces of considerable length the new head is localized at the anterior end of the piece because the axial gradient determines that the cells at this end are physiologically isolated to a much higher degree than the cells at the posterior end. A group of cells developing independently at a transverse cut surface gives rise to a head, but when developing in subordination to other parts, gives rise to a posterior end. In short pieces, biaxial heads, biaxial tails or reversal of polarity may occur according to the relations between the rates of the regions x , y and z .

6. The only logical conclusion from the data of experiment and observation is that a single fundamental reaction system is the basis of development and inheritance in each species, race or individual. The apical or head region, or the dominant part of that region, represents the fundamental reaction more nearly than any other part of the organism.

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TWO SEX-LINKED LETHAL FACTORS IN DROSOPHILA AND THEIR INFLUENCE ON THE SEX-RATIO

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SEVEN FIGURES

By a recessive lethal factor I mean any factor that brings about the death of the individual in which it occurs, provided its effect is not counteracted by the action of its normal allelomorph. The term is not intended to mean that some poison is produced that destroys the individual, but rather some defect is inherited that is serious enough to render the individual unable to live. The defect may be a physiological defect or a morphological malformation; in fact, a defect in any organ essential for the life of the larva, pupa, or imago would come under the general category of lethal. In the present case I have not attempted to discover where the elimination of the individual occurs, or to what specific defect it is due.

Lethal factors may be sex-linked or not, i.e., they may be carried by the sex chromosome or by an autosome. If the lethal factor is sex-linked it will kill any male in which it occurs, since the male has but one X chromosome. Such a factor can, therefore, never be transmitted through the male line, and, as a consequence, it is not possible to transfer a lethal factor from one lethal stock to another lethal stock, and in this way get two lethals together, because, as we have seen, all males that contain a sex-linked lethal factor die. If two sex-linked lethals should ever occur in the same female one must have arisen as a mutation independently of the other.

THE FIRST LETHAL FACTOR

The origin of this stock has already been described (Rawls,¹ Morgan,²). The first part of the data that follow includes those already given in my former paper. At the time when that paper was written the 'returns' were still coming in. Most of the counts here given for the first lethal were made by Mr. J. S. Dexter who was assisting me at the time.

Females from the lethal stock sent to me by Miss Rawls were mated to white-eyed males in pairs. Some pairs gave 1:1 ratios, i.e., equality in the sexes; and others gave 2:1 ratios, i.e., two daughters to one son. Those mothers that gave as many sons as daughters (e.g., pairs OM and K) were not heterozygous for lethal, and all their offspring should continue normal. Those mothers (e.g., pair L) which gave only half as many sons as daughters should be heterozygous for the lethal factor; and the missing sons should be those that received this lethal bearing sex-chromosome from their mother.

To bring out this difference clearly, daughters (heterozygous for white) from the normal lines OM and K were mated to white males. That they did not carry the lethal factor is shown in table 1 by the production of a 1:1:1:1 proportion, which is that normally expected from a cross between a white male and a red female heterozygous for white.

In contrast to the results of table 1 are those from the mating of daughters (heterozygous for white) from the pair L, which gave a 2:1 ratio, to white males. The results show that while half the daughters of pair L gave a normal sex-ratio (table 2), the other half gave the lethal sex-ratio of 2:1 (table 3).

The explanation of the fact that half of the daughters of L gave lethal sex ratios, and half gave normal ratios is that their mother was heterozygous for lethal. Half of her gametes were lethal and half non-lethal, so that of her daughters, half again should be heterozygous for lethal and half free from lethal.

¹ Biological Bulletin, vol. 24, 1913.

² Science, vol. 36, pages 718-20, 1912.

A comparison of tables 2 and 3 brings out the very striking fact that all the lethal sex ratios are characterized by the almost complete disappearance of a particular class of males—the red-eyed males, and that the absence or the smallness of this class is a *surer* index of lethal cultures than is the sex-ratio itself! Just as the red male class is almost exterminated, the white male class is almost undiminished, i.e., it is of about the size of each female class. That it is not nearer in size to the female class is due to the relatively poorer viability of these males.

This disproportionate effect of the lethal upon the size of the two male classes is due to association, i.e., the loci occupied respectively by the lethal and the white factors are very *close together* in the sex-chromosome. It would require a break of the chromosome between these two points to produce a red male which lives (non-lethal) or a white male which dies (lethal), and

TABLE 1

The offspring of twelve red-eyed daughters of O M, and of six red-eyed daughters of K when mated separately to white-eyed males

	RED ♀♀	WHITE ♀♀	RED ♂♂	WHITE ♂♂
O M 1.....	41	33	34	28
O M 2.....	21	21	13	20
O M 3.....	9	16	10	10
O M 4.....	30	29	44	34
O M 5.....	24	17	24	19
O M 6.....	13	11	14	11
O M 7.....	31	21	28	17
O M 8.....	24	25	22	26
O M 9.....	11	7	13	13
O M 10.....	12	8	8	8
O M 11.....	10	12	6	7
O M 12.....	11	16	16	23
K 1.....	45	37	54	41
K 2.....	38	38	36	38
K 3.....	39	37	34	35
K 4.....	55	33	46	56
K 5.....	42	40	45	44
K 6.....	8	13	9	15
Totals.....	464	414	456	445

TABLE 2

The offspring of red-eyed daughters of pair L, which when mated to white-eyed males, gave 1:1 sex-ratios

	RED ♀♀	WHITE ♀♀	RED ♂♂	WHITE ♂♂
L 1.....	32	47	34	32
L 2.....	36	38	30	31
L 3.....	13	25	18	24
Dup.* L 3.....	24	18	31	26
L 6.....	52	41	43	34
Dup. L 6.....	30	20	33	23
L 10.....	2	2	3	1
L 12.....	19	12	17	8
L 15.....	75	10	64	5
L 16.....	26	20	21	21
L 17.....	38	28	23	26
Dup. L 17.....	11	7	8	14
L 19.....	63	64	54	50
Dup. L 19.....	24	16	9	14
L 22.....	24	29	27	23
L 25.....	68	50	44	51
Dup. L 25.....	40	30	45	42
L 28.....	32	35	36	34
Dup. L 28.....	5	1	3	5
L 31.....	30	44	36	33
Dup. L 31.....	24	18	16	19
L 33.....	22	18	18	25
Totals.....	690	573	613	541

* The cases marked 'Dup.' refer to a second lot of offspring produced by the female of a given number.

it is assumed that breaking and crossing-over is infrequent in proportion as these loci are close together.

These relations can be represented by means of the following analysis and by figure A. Let X stand for the sex differentiator, l for the recessive lethal factor and L for its dominant allelomorph, w for the factor which determines white eyes and W for its normal allelomorph. The analysis for the cross of the female heterozygous for lethal and the white-eyed male is as follows:

P ₁	Lethal-bearing red-eyed ♀ X l W — X L W	
	Normal white-eyed ♂ X L w — —	
	F ₁ females	F ₁ males
	X l W X L w red ♀ (A)	X l W — red ♂ (dies) *
	X L W X L w red ♀ (B)	X L W — red ♂ (lives)

TABLE 3

Red-eyed daughters of L, which when mated to white-eyed males, gave 2:1 sex-ratio.

	RED ♀♀	WHITE ♀♀	RED ♂♂	WHITE ♂♂
L 4.....	0	7	0	7
L 5.....	2	3	0	1
Dup. L 5.....	1	1	0	0
L 8.....	50	43	1	28
Dup. L 8.....	48	46	0	31
L 9.....	48	63	0	19
Dup. L 9.....	13	14	1	5
L 18.....	36	53	0	45
Dup. L 18.....	3	8	0	4
L 20.....	30	21	0	21
Dup. L 20.....	14	19	0	22
L 23.....	17	15	0	15
Dup. L 23.....	14	19	0	22
L 24.....	48	47	0	58
Dup. L 24.....	23	14	0	17
L 26.....	11	10	0	14
Dup. L 26.....	6	3	0	2
L 32.....	4	7	0	3
L 34.....	29	23	0	20
Dup. L 34.....	36	41	0	36
Totals.....	433	457	2	370

There are two classes of daughters (A and B) alike in appearance, but one of them (A) is heterozygous for lethal. The offspring of half the daughters (B) should be, as we have seen in table

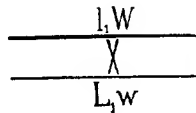


Figure A

2, non-lethal, and of the other half (A) lethal-bearing. Table 3 is the result of the mating of such daughters (A) heterozygous for lethal and white to white males.

In order to represent in a diagram what takes place in (A) let the pair of sex-chromosomes of the F_2 female be drawn in two

parallel lines; the upper, heavier one, the maternal, and the lower, lighter, one the paternal chromosome. Lethal and non-white entered from the mother and non-lethal and white from the father. The sex-chromosomes of the F_1 female are represented with the loci for lethal and white close together. The cross between the lines indicates that crossing-over occurs between the loci in question. (The cross does not represent the postulated twisting, but only that it occurs in this general region.) Although for convenience the cross stands midway between the loci this is only a convention, for it will be understood that crossing over occurs at any point between the loci in question. The gametes of A will be of four classes, the first two, where no break has occurred between the loci L and W; and the second two, where a break has occurred, and crossing over has taken place. The first class of gametes (non-cross-overs) will be of two kinds, equally numerous, viz., l W and L w. The second class of gametes (cross-overs) will also be of two kinds, viz., l w and L W, but relatively few in number.

	$F_1 \text{ } \varnothing$		$X \text{ l W } X \text{ L w}$
	White σ^7		$X \text{ L w } \sigma^7$
Gametes of F_1	$X \text{ l w} - X \text{ l W} - X \text{ L w} - X \text{ L W}$		
	$X \text{ L w} -$		
	$F_2 \text{ females}$		$F_2 \text{ males}$
	$X \text{ l w } X \text{ L w}$ white		$X \text{ l w} -$ white (lives)
	$X \text{ l W } X \text{ L w}$ red		$X \text{ l W} -$ red (dies)
	$X \text{ L w } X \text{ L w}$ white		$X \text{ L w} -$ white (lives)
	$X \text{ L W } X \text{ L w}$ red		$X \text{ L W} -$ red (dies)

The analysis given above shows that rarely a red female should occur which is not heterozygous for lethal. The chance for her occurrence is the same as for the occurrence of the red male that lives, viz., 2:370 (table 3) or 1:185. Since therefore over 99 per cent of the red females are heterozygous for lethal we have kept the stock running by mating in mass cultures the red-eyed females to their white brothers. The presence of a female homozygous for non-lethal would make itself apparent at once by the appearance of red males which otherwise are extremely rare, and the reappearance of the 1:1:1:1 proportion of table 2. In this case the stock could be recovered by breeding

a few red females individually, and continuing one of the lines which gives a lethal sex-ratio and no red males or only an occasional one. Records were made of the offspring of pairs from some of these mass cultures of stock, and table 4, which is comparable with table 3, shows by the small number of red males that none of the females used were cross-overs (B) free from lethal.

TABLE 4

Red-eyed females (A) carrying the lethal factor gave the following offspring when mated in mass cultures to their white-eyed brothers

	RED ♀♀	WHITE ♀♀	RED ♂♂	WHITE ♂♂
L 242.....	118	126	2	93
L 181.....	36	43	0	30
L 201.....	180	138	2	125
L 8 L.....	294	243	0	207
L 9 G.....	38	36	0	23
L 9 K.....	18	27	0	4
L 2011.....	47	36	0	39
L 9 K A.....	97	89	0	68
Totals.....	828	738	4	589

If the analysis of this case is correct, the very small 'cross-over' class of red males should be entirely normal and should be unable to transmit the lethal characteristic. One of these males (of L 9, table 3) was tested by mating him to a wild female. There were produced 61 daughters and 48 sons, which is a fair approximation of the 1:1 ratio expected whether the father were lethal or not. But if the father were a male which carried the lethal, and by some means survived (i.e., if lethal were not invariably fatal in its action) then *all* these 61 daughters should be heterozygous for lethal and should give lethal ratios in F_2 . The F_2 generation was raised and consisted of 440 females and 377 males which is undoubtedly a non-lethal ratio.

Another of these red-eyed males (from L 242, table 4) was tested by mating to his red sisters heterozygous for lethal and white (type A). The F_1 consisted of 59 red females and 24 white males. The absence of red sons shows that the mother was of the type supposed, and the fact that there were twice as

many females as males shows that the male was non-lethal; for, had he been lethal, half the daughters of this cross would have been homozygous for lethal and would not have appeared. This would have resulted in equality of red females and white males. The test of this male was continued into F_2 by breeding three F_1 pairs, which gave:

	red ♀ ♀	white ♀ ♀	red ♂	white ♂
L 242 AA.....	25	0	12	0
L 242 AB.....	34	0	20	0
L 242 AC.....	12	11	7	19

This result is in conformity with the view that the grandfather was non-lethal, and impossible on the view that he was lethal. On the first view there should be two kinds of daughters (exclusive of cross-overs), viz., those heterozygous for lethal and free from white which would give a 2:1 sex-ratio, and those free from lethal but heterozygous for white (type B) which should give a 1:1:1:1 proportion. Fortunately both types were met with among the three daughters selected at random.

Theoretically, none of the white F_2 females in the cross analyzed should be heterozygous for lethal, except those rare cases due to crossing-over. These should be as frequent as the cross-over males, viz., one in about two hundred. Of the ten granddaughters tested, nine gave (table 5) 1:1 ratios, but one (L 9 D) gave apparently a 2:1 sex-ratio, although it was not seen in time to test further. Such a female mated to a red male in P_1 and the daughters again mated to red males should give results in many ways the converse of those obtained in tables 3 and 4, for, now, the red males would live and the white males die.

The locus of Lethal I

In table 3 the number of cross-overs (red males) was two, and of non-cross-overs (white males) was 370. Table 4 gives 4 cross-overs to 589 non-cross-overs. The total of 6 cross-overs to 959 non cross-overs gives the percentage of crossing-over between the loci lethal and white as 0.6 per cent.

TABLE 5

Offspring of white-eyed daughters of lethal flies mated in pairs to white-eyed males

	WHITE ♀♀	WHITE ♂♂
L 9 D.....	39	17
L 241.....	100	100
L 9 F.....	44	30
L 202.....	136	121
L 2421.....	64	56
L 2424.....	14	19
L 2425.....	37	30
L 2426.....	42	54
L 2427.....	32	30
L 2428.....	24	17
Totals.....	532	474

On the scheme of locating the factors that we have employed, 0.6 per cent of crossing-over means that lethal lies at a distance of 0.6 of a unit from white, but in which direction from white is not so far shown. In order to determine on which side of white the lethal lies, it is necessary to know the distance between lethal and another known point of the chromosome. The second point chosen was the factor for miniature wing. Two daughters (type A, from L 9) were mated to eosin miniature³ males and gave the following F₁ offspring (table 6).

TABLE 6

	RED LONG ♀♀	WHITE-EOSIN LONG ♀♀	RED LONG ♂♂	WHITE LONG ♂♂
L 93.....	9	7	0	6
L 94.....	30	28	0	21
Totals.....	39	35	0	27

The red-eyed, long-winged daughters (from table 6) were mated to their white-eyed, long-winged brothers with the following F₂ results (table 7).

³ Eosin is allelomorphic to white, so that any linkage results given by white or by eosin have the same value, and can be combined. The female which is white-eosin in composition is distinguishable from the pure eosin.

TABLE 7

	RED LONG ♀	WHITE-EOS LONG ♀	EOSIN MIN. ♂	RED LONG ♂	EOSIN LONG ♂	RED MIN. ♂
L 93 B.....	256	250	118	0	90	0
L 93 B.....	94	70	54	0	24	0
L 94 a.....	128	102	64	1	47	0
L 94 a.....	59	56	45	0	35	0
L 94 c.....	215	182	123	0	82	0
Totals.....	752	660	404	1	278	0

In the above F_2 results the proportions of the males give a measure of the amount of crossing-over between the lethal and the white loci. The non-cross-overs number $404 + 278$ or 682. The data from the males of tables 3 and 4 bear on this same point. The combined data give $2 + 4 + 1$ or 7 for the number of cross-overs in comparison with $370 + 589 + 682$ or 1641 for the number of non-cross-overs with respect to the interval L-W. This gives 0.4 as the percentage of crossing-over.

The data (table 7) of the cross in which miniature is involved give as the percentage of apparent crossing-over between lethal and miniature 41 which is somewhat higher than the value derived from our other data for this case.

Similarly the white-eosin daughters (cf. table 6) were mated to their white-eyed, long-winged brothers and gave:

	wh.-eos. long ♀	white long ♀	white long ♂	eosin min. ♂	white min. ♂	eosin long ♂
L 93 A.....	126	143	48	61	43	28
L 93 D.....	69	52	46	37	23	25
	195	195	94	98	66	53

The white-eosin daughters that gave the above results should rarely contain lethal (because of linkage) and the result 1:1 confirms the expectation. Two classes of males, viz., white miniature and eosin long are smaller than the other male classes because they are due to crossing-over between the white-eosin and miniature loci.

The most convincing evidence bearing upon the order of these factors in the linear series is that derived from a consideration

of the class whose occurrence would necessitate a double cross-over. A double cross-over class is always much less likely to be represented than any single cross-over class. Inspection of table 7 shows that the class red miniature is the only one not represented. The order of loci which would make the red miniature the double cross-over class is lethal, white, miniature (L W M). If lethal were not outside of the white to miniature section of the chromosome (that is, if the order of factors were white, lethal, miniature, W L M) the double cross-over male class would be the red, long class. The fact that this class is represented argues against the probability of white, lethal, miniature as the order of factors and points to lethal, white, miniature as the correct order.

A confirmation of lethal, white, miniature as the order of factors was obtained by using yellow and miniature as the fixed points instead of white and miniature. A large amount of data has shown that yellow is about 1.1 units from white on the side of white away from miniature. The data just given show that lethal lies at a distance of 0.4 units from white and probably on the same side from white as does yellow. *Lethal should lie, therefore, between yellow and white at a distance of 0.7 from yellow and 0.4 from white.*

Sturtevant made the following mating (not published). A gray, lethal long-winged female was mated to a yellow miniature male. They produced 169 gray red-eyed females and 64 gray white-eyed males. The female used was, as shown by the sex-ratio, heterozygous for lethal and for white. Of her daughters, half should be heterozygous for lethal and should not bear white, while the other half should be heterozygous for white, but should not bear lethal.

Six females of the latter type crossed individually to their white brothers gave non-lethal F_2 ratios in which white males and females occurred. Six other females gave the following lethal ratios in which no white appeared (table 8).

TABLE 8

GRAY LONG ♀	YELLOW MINIATURE ♂	GRAY MINIATURE ♂	YELLOW LONG ♂	GRAY LONG ♂
53	10	0	5	0
58	19	1	9	0
63	14	0	9	0
87	21	0	6	0
47	9	0	9	0
67	13	0	6	0
375	56	1	44	0

If lethal is between yellow and miniature the double cross-over class is gray long. The data of table 8 shows that this is the class not represented in the males, and the result is in harmony with the view that the order of factors is yellow, lethal, miniature. Furthermore, these data give us the distance of lethal from yellow $\frac{1}{1.1}$ or 0.7, which is the expectation if lethal lies between yellow and white and at a distance of 0.4 from white ($1.1 - 0.4 = 0.7$). Both experiments taken together show by the agreement in linkage, and in the absence of particular classes that the chromosomal line-up is yellow, lethal, white, miniature. This linkage of 0.4 is the strongest met with so far, corresponding to a 'gametic ratio' of about 250:1.

The reappearance of Lethal I

In 1914 a cross was made between yellow white normal females and gray red abnormal males. The cross gave in the F_1 generation yellow white males and abnormal females. No disturbance of the sex-ratio was noted, although it was not looked for specifically. Thirteen F_1 matings in pairs were made. Three of these pairs gave a sex-ratio of two females to one male.

<i>females</i>				<i>males</i>			
gray red	yel. white	gray white	yel. red	gray red	yel. red	gray white	yel. white
70	69	0	1	73	1	0	0
56	53	0	1	51	0	0	0
54	54	0	0	46	1	0	0
180	176	0	2	170	2	0	0

Furthermore, there were no yellow white males in the 2:1 ratios, although as many are expected as gray red, unless some disturbance is present. In fact, in the other ten pairs as seen below, the number of males in these two contrary classes correspond numerically (gray red, 649; yellow white, 621). The absence of the yellow white males aroused a suspicion that a sex-linked lethal was present, close to the yellow and the white factors.

<i>females</i>				<i>males</i>			
gray red	yel. white	gray white	yel. red	gray red	yel. red	gray white	yel. white
707	687	5	10	649	9	5	621

About a dozen of the yellow white females (from the lot giving 2:1 ratios) were bred to eosin males (stock). Only four pairs produced offspring. The records are as follows:

	white eosin ♀	yellow white ♂
B ₁	11	9
G ₁●.....	25	5
G ₂	26	14
C ₁	36	21

The twelve pairs were afterwards transferred to one bottle and gave as a mass result:

White-eosin ♀ ⁴	Yellow white ♂
101	33

Matings (in pairs) were made between virgin females from B₁, G₁, G₂, C₁, and also some from the mass cultures with eosin males of stock. The results are given in table 9, where it will be seen that there are two kinds of females—some giving a 1:1 ratio (with the yellow white male class fully represented) and others giving a 2:1 ratio (with the yellow white male class entirely absent). Of the females tested 13 were non-lethal bearing, and 10 were lethal bearing. This is the expectation if the 2:1 ratio is due to a sex-linked lethal. If we return to the original series B₁, G₁, G₂, C₁ we find that only one daughter of B₁ was tested. She was non-lethal bearing. Probably B₁ was non-lethal as the

⁴ Also one mosaic.

ratio 11:9 indicates. The other three females, G_1 , G_2 , C_1 were lethal bearing as their ratios had indicated.

An examination of the male classes of the lethal lots shows that the lethal factor in question is the original lethal I that has reappeared, now linked to white instead of to red as originally. As a consequence the lethal males are now the white males, while before they were the red males. The following calculations will substantiate these statements.

The yellow white females, that carry the lethal, received from their mother a sex-chromosome carrying yellow (y), lethal (l_1), white (w) factors (upper line, fig. B); and from their father a

TABLE 9

	FEMALES		MALES			
	eosin	white-eosin compound	gray eosin	yellow eosin	gray white	yellow white
B_1	38	49	54	0	0	46
C_1	49	72	52	1	0	61
	64	51	61	0	1	83
	99	52	78	1	2	80
	91	90	87	1	0	78
	43	46	45	1	0	47
G_1 a	60	45	40	0	0	45
G_1 b	68	58	62	0	0	72
G_1 c	63	62	75	1	2	56
G_1 d	64	58	54	0	0	39
G_2 a	40	41	47	0	0	50
G_2 b	87	77	72	0	2	60
G_2 c	98	110	85	1	1	73
Total	864	811	812	7	8	760
C_1 a	27	41	47	1	0	0
C_1 b	59	70	76	0	0	0
C_1 c	89	76	86	1	0	0
G_1 a	52	75	59	1	0	0
G_1 b	35	50	46	0	0	0
G_1 c	83	82	61	1	0	0
G_1 d	72	81	60	0	0	0
G_2 a	103	103	96	0	0	0
G_2 b	77	86	72	0	0	0
G_2 c	13	16	9	0	0	0
Total	660	738	668	4	0	0

sex-chromosome carrying the factors gray (Y) non-lethal (L_2) and eosin (w^e). The two non-cross-over classes, the four single

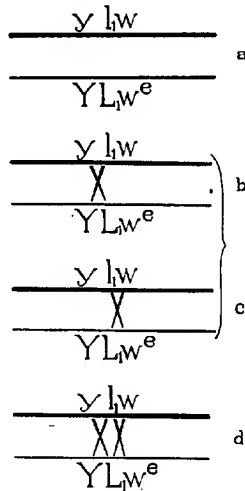


Figure B

cross-over classes, and the two double cross-over classes of gametes are as follows (see also fig. B).

Non-cross-overs.....	$y\ l\ w$ —(dies) $Y\ L\ w^e$ —gray eosin $y\ L\ w^e$ —yellow eosin $Y\ l\ w$ —(dies)
Single cross-overs.....	$y\ l\ w^e$ —(dies) $Y\ L\ w$ —gray white $y\ L\ w$ —yellow white $Y\ l\ w^e$ —(dies)
Double cross-overs.....	$y\ l\ w^e$ —(dies) $Y\ l\ w^e$ —(dies)

If we take the sum of all the male classes (668 + 4) and divide into the single cross-over class of flies (4) we get a percentage of 0.6 to represent the distance of the new lethal from yellow. This corresponds with the calculated percentage of the original lethal which is 0.7. In other words, this lethal lies near yellow and white—somewhat nearer to white. The single cross-over

between yellow and lethal occurs four times and the other cross-over, viz., that between lethal and white (to give gray white males) is not realized at all. The sons of the non-lethal sisters involve only two pairs of characters, hence there are two contrary single cross-over classes, viz., yellow eosin males and gray white males. These are expected in equal numbers as is practically realized (7YR and 8GW).

The data for non-lethals permits us to calculate again the distance of yellow from white (or eosin). The sum of all the male classes ($812 + 760 + 7 + 8 = 1587$) into the sum of the two cross-over classes ($7 + 8 = 15$) gives a percentage of .95 which is very close to the value (1.1) given by other experiments.

The question naturally suggests itself as to whether the re-appearance of lethal I was due to mutation, or to descent from a common original stock. The stock used by Miss Rawls ('12) was wild stock that had been in the laboratory about a year when she found her high ratios running in it. Now the yellow stock, the white eyed stock, and the stock with abnormal abdomen had arisen before 1912, and had been maintained as pure stock. Hence the new lethal appears to have arisen as an independent mutation. Contamination is excluded on the grounds that it is well nigh inconceivable that a lethal could be inserted between yellow and white by a double cross-over, after contamination in the yellow white stock.

Summary of linkage data of Part I

From the preceding pages the data bearing on the linkage values have been collected and may here be summarized. In the following table for each linkage value the total number and the number of cross-overs is given; from which the percentage of crossing-over is calculated and is given in the last column:

	total	cross-overs	per cent cross-overs
Yellow lethal.....	1015	7	0.7
Lethal white.....	2559	7	0.27
Yellow white.....	5564	52	0.9
Yellow miniature.....	131	45	34.0
Lethal miniature.....	814	323	40.0
White miniature.....	994	397	40.0

THE SECOND LETHAL FACTOR

In an experiment with certain stock which had been inbred for three years, a pair produced:

73 females and 16 males (= 5:1)

These numbers represent the total output of this pair, or at least all the flies that were produced from one bottle. (A) Twenty-two of the seventy-eight females were mated to white miniature (of which two pairs produced nothing). (B) Twenty-nine of the seventy-eight females were mated to eosin vermilion males (of which six pairs produced nothing). (C) Twenty-four females of the seventy-eight were mated to eosin miniature males (of which four pairs produced nothing). The experiments with white and with eosin should give similar results, since white (w) and eosin (w^*) are allelomorphs.

The sixteen males were mated to eosin miniature, white miniature, and eosin vermilion females, but every one of these males proved sterile.

The output of the three lots of females mated to the respective males mentioned above is shown in A, B and C of table 10. Under each column, A, B, C, the progeny is provisionally classified, first, into those that give approximately, a 1:1 ratio, and then those that gave approximately a 2:1 ratio (or higher). A horizontal line separates these two classes.

The classification into these two groups may appear arbitrary, since the ratios in the two classes come very near to each other in some cases. For the present it will suffice to explain that the 2:1 ratio is expected if one lethal is present, the 1:1 ratio if no lethal is present.

From table 10 one can get no clue as to the location of the lethal factor that is assumed to be upsetting the normal sex-ratio, but by breeding the females to a male that carries the same sex-linked factors as their fathers, the location of the lethal factor is revealed.

Crosses with white miniature males

A number of daughters were taken from No. 12 (indicated by a star in table 10) and bred in pairs to white miniature males. Since No. 12 was carried further, I have selected it first for illustration.

The data are arranged in table 11, under two headings, viz., those pairs that gave a sex-ratio of 1:1, and those that gave 2:1. The data from pairs with a 1:1 ratio show nothing except the association of white and miniature. The data from the 2:1 ratio, in which the second lethal is present, show no males in the

TABLE 10

A				B				C			
NO.	FEMALES	MALES	SEX RATIO	NO.	FEMALES	MALES	SEX RATIO	NO.	FEMALES	MALES	SEX RATIO
2	34	51	.7	II	60	44	1.4	a	30	40	.8
3	66	51	1.3	VII	53	44	1.2	b	76	56	1.4
5	50	41	1.2	IX	7	8	.9	h	13	12	1.1
6	64	45	1.4	X	14	18	.8	j	54	53	1.
8	77	56	1.4	XI	63	64	1.	m	24	20	1.2
9	69	49	1.4	XII	58	56	1.	p	67	57	1.2
13	55	41	1.3	XIV	71	50	1.4	q	38	34	1.1
16	71	53	1.3	XV	24	29	.8	r	65	47	1.4
17	64	48	1.3	XVI	59	47	1.2				
18	78	63	1.2	XVIII	90	83	1.1	c	58	35	1.7
				XIX	67	56	1.2	d	67	29	2.3
1	97	43	2.3	XX	44	33	1.3	e*	116	46	2.5
4	70	35	2.	XXI	57	52	1.1	f	124	22	5.4
7	72	24	3.	XXIII	50	40	1.2	g	68	21	3.2
10	59	38	1.5					i	53	19	2.8
11	47	25	1.9	I	79	37	2.1	k	62	30	2.
12*	122	32	3.8	III	114	48	2.4	l	80	31	2.8
14	64	26	2.5	IV	64	26	2.4	n*	58	20	2.9
15	78	34	2.3	V	74	31	2.5	o	65	28	2.3
19	86	38	2.3	VI	16	5	3.2	s	77	31	2.5
20	55	34	1.6	VIII	68	33	2.1	t	10	3	3.3
				XIII	53	26	2.				
				XVII*	96	30	3.2				
				XXII	21	14	1.5				

red-long class, which is the lethal class and, as will appear, also the double cross-over class.

One of the single cross-over classes is red miniature; the other, white long. Both are smaller than the non-cross-over class, white miniature.

TABLE 11
(From No. 12 of table 10)

NO.	RED LONG ♀	WHITE MIN. ♀	RED MIN. ♀	WHITE LONG ♀	RED LONG ♂	WHITE MIN. ♂	RED MIN. ♂	WHITE LONG ♂	SEX RATIO
1	43	24	13	6	28	31	8	20	1.
3	20	12	5	3	18	19	10	13	.7
7	48	31	7	10	7	26	9	4	2.1
8	37	37	18	15	23	39	23	9	1.2
9	33	26	13	8	22	27	15	8	1.1
13	33	34	10	14	22	21	11	13	1.4
15	25	18	13	16	18	23	13	6	1.2
18	27	26	14	16	38	33	13	20	.8
19	24	32	26	10	10	27	4	4	2.
21	39	32	19	18	12	35	19	6	1.5
22	13	25	5	6	24	6	9	13	1.
26	26	25	13	22	9	20	22	5	1.5
29	24	22	6	13	14	25	5	11	1.2
Total	392	344	162	163	245	332	159	132	
2	31	35	15	10	0	20	3	14	2.5
4*	28	24	16	16	0	19	3	7	2.9
5*	49	55	14	20	0	49	1	6	2.5
6	27	14	10	12	0	19	3	8	2.1
11	26	16	10	11	0	11	3	5	3.3
12	27	28	13	22	0	31	5	8	2.
14	23	28	5	9	0	29	3	9	1.6
16*	23	25	8	9	0	16	3	0	3.4
17	25	39	23	14	0	27	3	6	2.8
20	25	34	15	17	0	31	2	0	2.8
23	38	34	18	19	0	35	3	11	2.
24	34	24	14	10	0	31	5	7	1.9
25	21	31	13	10	0	19	2	2	3.3
27	25	27	5	8	0	17	3	4	2.7
28	26	23	12	8	0	23	3	5	2.2
30	29	27	20	17	0	33	5	6	2.1
31	31	20	11	14	0	26	4	6	2.1
Total	488	488	223	226	0	436	54	104	

In each generation the females that gave the highest sex-ratio were selected to breed from. Whatever disadvantage this method of selection may have, it at least makes more probable the retention of a 2:1 ratio.

TABLE 12
(From No. 16 of table 11)

NO.	RED LONG ♀	WHITE MIN. ♀	RED MIN. ♀	WHITE LONG ♀	RED LONG ♂	WHITE MIN. ♂	RED MIN. ♂	WHITE LONG ♂	SEX RATIO
a	46	25	10	24	0	30	7	10	2.2
b*	53	32	26	19	0	36	9	1	2.7
c*	47	49	22	19	0	26	6	4	3.8
d	37	34	17	11	0	29	3	5	2.7
e	35	37	25	19	0	29	2	4	3.3
f	31	30	10	15	0	20	2	2	3.6
g	55	49	20	28	0	66	9	14	1.7
h	43	51	25	15	0	40	6	1	2.8
i	20	20	9	9	0	14	4	4	2.6
Total	367	327	164	159	0	290	48	45	

TABLE 13
(From No. b of table 12)

NO.	RED LONG ♀	WHITE MIN. ♀	RED MIN. ♀	WHITE LONG ♀	RED LONG ♂	WHITE MIN. ♂	RED MIN. ♂	WHITE LONG ♂	SEX RATIO
1	35	28	9	12	0	19	6	3	3.
2	35	39	10	18	0	36	2	11	2.1
3	39	32	24	19	0	29	2	4	3.1
4	30	37	18	10	0	31	6	1	3.1
5	36	44	21	6	0	29	4	0	2.2
6	36	36	16	8	0	38	5	1	2.2
7	44	26	22	14	0	35	3	10	3.
8	46	49	25	25	0	44	1	3	2.3
9	43	34	9	6	0	35	4	1	2.3
10	39	15	34	6	0	24	4	2	3.1
11	38	33	15	18	0	45	4	1	2.1
12	39	37	27	20	0	29	10	10	2.5
Total	467	410	230	162	0	394	51	47	2.6

From lot 16 of table 11 several virgin long-red females were mated in pairs to white miniature males. Their offspring are shown in table 12. Each pair shows in its offspring that the second lethal was present in the long winged red mother. A further generation was reared in pairs from red females of lots *b* and *c* of table 12. The offspring recorded in tables 13 and 14 show that the 2:1 sex-ratio continues.

Returning to table 11 there are records of two lots taken respectively from No. 4 and No. 5. The offspring from these pairs are here shown in tables 15 a and 15 b. Both show that lethal II is present as indicated by the absence (or rarity) of the red long males.

Before examining the preceding tables 10 to 15 in detail it may be helpful to have in mind certain theoretical relations. The location of factors for white (*w*) and for miniature (*m*) have been already determined. If we are dealing here with a lethal factor, it is essential to determine its position in relation to white and to miniature. For reasons that will appear later it is here assumed that this lethal lies between white and miniature (fig. C).

TABLE 14
(From No. c of table 12)

NO.	RED LONG ♀	WHITE MIN. ♀	RED MIN. ♀	WHITE LONG ♀	RED LONG ♂	WHITE MIN. ♂	RED MIN. ♂	WHITE LONG ♂	SEX RATIO
1	27	27	14	26	0	39	5	3	2.
2	26	35	6	15	0	23	7	5	2.3
3	53	59	30	32	0	56	7	2	2.7
4	61	49	39	13	0	44	7	3	3.
5	40	28	16	16	0	30	2	5	2.7
6	29	35	7	15	0	26	3	7	2.1
7	46	41	29	18	0	50	6	9	2.3
8	37	40	19	16	0	32	10	7	2.3
9	44	37	28	21	0	34	6	4	3.
11	59	55	27	25	0	67	8	7	2.2
12	16	10	1	21	0	23	3	1	1.8
13	41	34	11	11	0	31	5	3	2.5
Total	479	450	227	229	0	455	69	56	2.4

We are concerned with the heterozygous females only. Of the two sex chromosomes of these females, the one of maternal origin is indicated by the heavier line in the diagram (fig. C), the other, of paternal origin, is indicated by the lighter line in the figure. In the present case there entered from the maternal side the three factors: normal (red) eye (W), the second lethal (l_2), and normal wings (M); there entered from the paternal side the three factors: white eye (w), the normal allelomorph of lethal (L_2), and miniature wing (m).

In the case of this lethal the possibilities of interchange of factors between these chromosomes are shown in figure C, at b , c , d . If the crossing-over takes place between white and lethal, the case is indicated in (b). The two gametes that result from this single cross-over are WL_2m and wl_2M ; the latter never becomes realized in the male because he carries the lethal (l_2). Similarly, if the crossing over takes place between lethal and miniature the two gametes that are produced are WL_2m and wL_2M , of which the former carries the lethal factor. There is another possibility, viz., the double crossing-over (d).

TABLE 15a

(From No. 4 of table 2)

NO.	RED LONG ♀	WHITE MIN. ♀	RED MIN. ♀	WHITE LONG ♀	RED LONG ♂	WHITE MIN. ♂	RED MIN. ♂	WHITE LONG ♂
a	26	22	14	12	0	23	3	6
b	23	19	11	13	1	12	3	8
c	34	39	21	18	0	28	10	8
d	30	24	12	16	2	15	5	9
Total	113	104	58	59	3	78	21	31

TABLE 15b

(From No. 5 of table 2)

NO.	RED LONG ♀	WHITE MIN. ♀	RED MIN. ♀	WHITE LONG ♀	RED LONG ♂	WHITE MIN. ♂	RED MIN. ♂	WHITE LONG ♂
b	16	16	6	8	0	11	1	1
c	34	30	8	8	0	26	5	6
Total	50	46	14	16	0	37	6	7

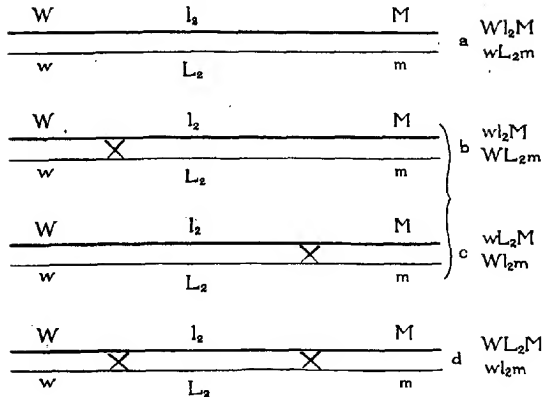


Figure C

In this case there is crossing over between L_2 and W and simultaneously between L_2 and M . Expressed in another way this means that the chromosomes cross between these two points, and the parts on the same side reconnect at the place of crossing. As a result of double crossing-over, the two gametes that result are WL_2M and wl_2m , the latter of which never appears in the males.

If a female with these possibilities is crossed to a white miniature male (i.e., a male that carries these two recessive characters) the male classes will correspond to the gametic classes of that female except for those that carry the lethal which are not realized. The most frequent gametic class will be the non-cross-over class. The frequency of the other classes will depend on the distance of the factors involved from each other, because the chance of the crossing of the chromosomes taking place will be in proportion to the interval between the two loci. For this second lethal factor, therefore, the surviving classes of males are the following:

Non-cross-over.....	$w L_2 m$ —white miniature σ
Single cross-over.....	$W L_2 m$ —red miniature σ
Single cross-over.....	$w L_2 M$ —white long σ
Double cross-over.....	$W L_2 M$ —red long σ

Lethal and eosin vermilion

Twenty-nine females of the original 78♀♀ to 16♂♂ ratio were bred to eosin vermilion males and gave the ratios recorded in table 10 under I-XXIII. Some of these females from one culture No. xvii, with a ratio of 98:30, were again bred to eosin vermilion. Their offspring are shown in the record of pairs of table 16. Ten pairs gave a 1:1 ratio, and eleven a 2:1 ratio. As in the case of the cross with white eyes it is the red-eyed class of males, the lethal and also the double cross-over class which is deficient. (One record *jj* with three cross-overs may be due to contamination since the three occurred in the same bottle and in the same count).

TABLE 16
(From No. xvii of table 1)

NO.	RED ♀	EOSIN VERM. ♀	VERM ♀	EOSIN ♀	RED ♂	EOSIN VERM. ♂	VERM ♂	EOSIN ♂
		<i>No lethal.</i>		<i>Sex ratio</i>	<i>1:1</i>			
a	33	35	25	19	28	44	11	11
d	50	40	9	17	29	42	12	2
i	48	47	19	27	39	53	15	11
j	37	34	14	10	23	33	8	7
o	48	53	13	16	44	41	10	10
p	38	29	22	8	22	29	11	4
v	57	44	13	23	43	56	15	10
dd	34	31	12	12	22	39	15	11
ee	36	45	19	15	36	42	11	5
gg	47	49	22	19	35	41	12	7
Total	428	407	168	166	321	420	120	78
c	61	66	26	30	0	49	4	17
f	39	49	17	12	0	34	3	7
q	34	34	15	16	0	45	3	13
s	44	45	14	10	0	49	5	9
u	48	39	18	14	1	41	5	5
w	44	29	17	10	1	44	3	9
y	51	38	7	17	0	59	4	11
aa	47	39	6	11	0	47	5	13
hh	45	37	22	15	0	40	10	11
jj	58	37	13	34	3	29	2	12
ll	28	31	12	12	0	27	4	2
Total	499	425	167	181	5	464	48	109

From the lot *jj* (table 16) nine red-eyed females were bred to eosin vermilion males. The results show (in table 17) that the second lethal was present in the red-eyed females. In the lower part of the same table there are four cases recorded in which only vermilion and eosin vermilion offspring were produced. The sex-ratio 2:1 recurs. The results are undoubtedly due to four vermilion females (1, 5, 10, 14) from *jj* of table 10 having been taken instead of red females as intended.

From lot *c* of table 16 also seven red-eyed females were bred to eosin vermilion males. The results are given in table 18, which is similar to the preceding tables.

The sum of all the female classes that belong to the 2:1 division in tables 2 to 6 b and the sum of the corresponding males, is as follows:

		<i>females</i>			
red long	white min.	red min.	white long		
1964	1835	916	852		
		<i>males</i>			
3	1690	247	290		

The linkage shown in these results will be discussed later.

TABLE 17
(From No *jj* of table 16)

NO.	RED ♀	EOSIN VERM. ♀	VERM. ♀	EOSIN ♀	RED ♂	EOSIN VERM. ♂	VERM. ♂	EOSIN ♂
2	39	39	8	11	0	23	4	9
3	34	23	4	8	0	24	2	4
4	32	33	14	11	0	14	2	16
6	28	36	8	20	0	19	4	9
8	24	12	9	5	0	26	2	3
9	27	16	11	10	0	25	8	7
12	44	29	23	10	0	30	7	8
13	32	39	14	10	0	32	2	5
15	18	23	11	3	0	26	2	7
Total	278	250	102	88	0	219	33	68
1		40	36			39	1	
5		32	30			32	4	
10		39	41			24	8	
14		41	31			31	2	
Total		152	138			126	15	

TABLE 18
(From No. c of table 16)

NO.	RED ♀	EOSIN VERM. ♀	VERM. ♀	EOSIN ♀	RED ♂	EOSIN VERM. ♂	VERM. ♂	EOSIN ♂
1	47	25	19	14	0	34	5	10
2	53	42	13	9	0	38	8	5
3	40	35	18	17	0	28	8	11
4	48	28	13	16	1	36	7	13
6	28	31	13	10	0	32	4	2
8	19	14	16	6	0	26	3	4
9	29	36	15	12	0	25	8	5
Total	264	211	107	84	1	219	43	50

TABLE 19
(From No. c table 10)

NO.	RED LONG ♀	EOSIN MIN. ♀	RED MIN. ♀	EOSIN LONG ♀	RED LONG ♂	EOSIN MIN. ♂	RED MIN. ♂	EOSIN LONG ♂
6	35	29	16	14	31	28	11	7
8	24	22	12	14	22	19	6	10
9	19	24	12	11	25	26	10	15
10	28	20	10	12	31	24	12	11
14	22	15	10	6	16	23	11	10
16	20	12	9	9	27	15	12	14
17	11	11	9	4	8	12	12	1
18	24	29	12	12	20	16	17	9
19	33	21	8	11	18	26	14	14
21	22	22	8	12	25	23	8	8
24	45	38	17	21	28	23	19	13
26	12	13	6	4	5	8	6	8
27	18	26	13	10	19	22	12	7
Total	313	282	142	140	275	265	146	127
2	25	28	16	7	2	21	5	8
3	27	23	8	4	0	23	2	7
4	39	30	17	12	0	24	12	8
7	26	22	13	18	0	25	5	8
11	33	24	12	18	0	29	5	10
12	33	16	17	22	0	19	2	9
20	33	31	14	16	0	27	4	3
23	41	32	18	23	0	23	9	13
25	26	24	15	11	0	29	1	12
Total	283	230	130	131	2	220	45	78

Second lethal and eosin

The females of lot *e*, table 10, which gave a ratio of 116:46, were bred to eosin miniature males. As shown in table 19, fourteen of the females gave a 1:1 ratio, and nine a 2:1 (or higher) ratio. In the latter (2:1) class the double cross-over is the red long male. Two of these that appear must be due to a double cross-over. It is just possible, of course, that contamination may have occurred because these two males appeared in the same bottle and on the same day.

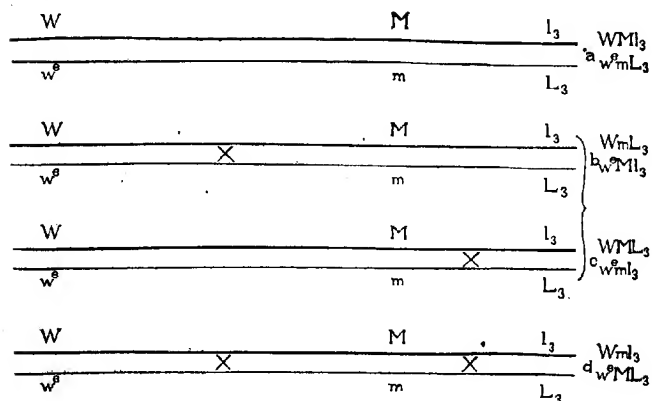


Figure D

The females of another lot, in table 10, viz., lot *n* with a ratio of 58:20, were also bred to miniature eosin males. The results shown in table 20 are strikingly different from any others so far obtained. The second lethal no longer appears to be present, yet in about half the cases the ratio is near 2:1. The question arises whether a new lethal has appeared, or whether we have in this set simply separated from the second lethal the other factor or factors that when combined with the second lethal gave the high 3:1 ratios.

No more light is thrown on this matter by the further history of this series. One lot only, viz., No. 39, seemed to give a 3:1

TABLE 20
 (From No. n of table 10)

NO.	RED LONG ♀	ROBIN MIN. ♀	RED MIN. ♀	ROBIN LONG ♀	RED LONG ♂	ROBIN MIN. ♂	RED MIN. ♂	ROBIN LONG ♂	SEX-RATIO
1	56	39	32	31	46	53	32	12	1.1
2	39	31	14	12	22	21	25	6	1.4
3	45	37	30	25	27	41	28	12	1.3
4	71	76	30	30	41	42	20	23	1.6
5	56	56	28	33	16	20	20	11	2.6
6	56	50	34	19	24	24	18	10	2.1
7	54	42	30	25	26	32	20	3	1.9
8	44	28	30	31	40	39	23	27	0.95
9	43	40	19	21	24	27	13	18	1.5
10	42	34	28	27	22	25	25	5	1.8
11	54	39	32	29	19	36	19	13	1.8
12	56	41	35	26	63	39	23	24	1.0
13	61	52	30	33	34	52	27	9	1.4
14	61	67	36	35	39	63	25	19	1.4
15	43	51	25	39	35	25	24	12	1.5
16	45	36	31	20	36	25	28	8	1.4
17	59	65	26	28	55	61	26	29	1.0
18	48	24	31	19	29	26	21	12	1.5
19	72	61	29	26	31	29	40	12	1.7
20	37	36	25	22	14	23	18	5	2.0
21	46	28	26	27	21	26	25	13	1.4
22	51	48	33	24	31	30	20	9	1.7
23	51	33	26	20	28	29	21	16	1.4
24	40	37	32	19	40	24	19	6	1.5
26	56	45	28	28	22	27	18	12	2.0
27	65	45	30	33	31	35	31	18	1.5
28	48	50	28	28	21	37	24	8	1.7
29	50	25	16	10	27	13	13	9	1.6
30	33	33	25	12	11	38	17	5	1.6
31	38	34	30	15	21	24	23	6	1.4
32	32	42	17	22	19	35	15	10	1.5
33	38	32	22	31	20	25	18	5	1.8
34	30	41	13	18	28	17	18	21	1.2
35	29	32	13	18	30	21	13	7	1.3
36	39	20	9	9	24	20	9	8	1.3
37	38	30	30	20	20	35	17	10	1.5
38	27	23	22	9	13	10	7	2	2.5
39*	49	39	16	13	22	1	0	10	3.3
40	33	31	5	10	21	15	11	4	1.5
41	20	18	18	7	11	14	7	0	1.2
42	19	21	11	6	13	10	9	5	1.5
Total.....	1874	1612	1025	913	1117	1189	809	454	

TABLE 21
(From No. n of table 10)

NO.	RED LONG ♀		EOSIN MIN. ♀		RED MIN. ♀		EOSIN LONG ♀		RED LONG ♂		EOSIN MIN. ♂		RED MIN. ♂		EOSIN LONG ♂		SEX RATIO
	RED	LONG ♀	EOSIN	MIN. ♀	RED	MIN. ♀	EOSIN	LONG ♀	RED	LONG ♂	EOSIN	MIN. ♂	RED	MIN. ♂	EOSIN	LONG ♂	
a	26		31		11		16		34		34		11		12		1:0.9
b	46		46		19		21		35		24		18		11		1:1.5
c	14		13		5		4		2		8		5		2		1:2.1
d	12		7		6		7		3		6		6		0		1:2.1
e	23		21		13		12		28		37		15		13		1:0.7
f	5		6		6		3		5		9		6		3		1:0.9
g	13		11		5		9		5		4		5		3		1:2.2
	139		135		65		72		112		120		66		44		

ratio and from this lot a few females were bred to eosin vermilion males. Their offspring are recorded in table 21. The data appear to verify the conclusion that the second lethal has gone while a disturbance of the sex-ratio remains. Can these results be interpreted to mean that the second lethal disappeared, and a third lethal, L_3 , that was present and gave the original high ratio of 78-16, has been retained?

If such a third lethal is here affecting the sex-ratio and if it lies beyond (to the right) of miniature as shown in figure F, then the deficient class is eosin long males. If the lethal kills then the only representative of that class will be double cross-overs and should be infrequent in proportion as L_3 is close to M. In fact, in a large number of cases in the table, the l_3 class, is behind the other male classes. In order, however, for the double cross-over class, eosin long, to be as frequent as appears in the table, the distance of L_3 to M would have to be very long indeed. In order to calculate this distance it would first be necessary to find out from table 20 those classes in which the factor occurs, but it is impossible to do this, for there are too many cases whose position is uncertain. If we make a single attempt to pick out such cases on the basis of the deficiency in the eosin long class we find that the most frequent class is that with a ratio of 1.5:1 and not 2:1 or more as would be the case if a lethal like the other two lethals is here present.

The meaning of the high sex-ratios that appeared in these cases must remain therefore unknown, and it is idle to speculate whether the results are or are not due to a non-sex-linked lethal, a sex-linked partial lethal, a sex-limited factor or factors, etc. The importance of following up this case was not appreciated at the time and this line was not continued except to breed seven individuals from No. 39⁵ (table 21).

Data from stock of Lethal II

The stock that carries Lethal II has been maintained by breeding long red females to white or to eosin miniature males from the regular eosin miniature stock. This has been continued to the present time. The results are shown in the next table, where,

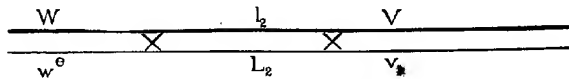


Figure E

through ten successive generations (bred in pairs), the inheritance of a 2:1 ratio has maintained itself. The horizontal lines in the first column separate generations; in some cases two or even three lines were tested, in each generation, in other cases only one. In all there are 13,749 flies recorded. The sex-ratio is 2.3:1. The order of the factors is shown in figure E. The percentage of cross-overs between white and lethal is 8.8, that between lethal and miniature is 16.3; and that between white and miniature is 24.7 which agree sufficiently with the percentages already given. The females are also available for cross-overs between white and miniature, and these give 28.3.

The linkage of Lethal II with white (or eosin) and with miniature

The diagram (fig. C) on page 103, will serve to recall the order in which the three pairs of factors enter. The F₁ female has received from her mother a sex-chromosome, bearing the factor for red (W) Lethal II (l₂) and long wings (M); and a sex-

⁵ No. 39 is peculiar in that eosin miniature and red miniature males are too few in numbers.

chromosome from her father, bearing the factors for eosin (w^*) normal or non-lethal (L_2) and miniature wings (m). The possible combination in the eggs of this F_1 female are repeated here:

Non-cross-over.....	$W l M$ —red <i>lethal</i> long $w^* L m$ —eosin miniature $W L m$ —red miniature $w^* l M$ —eosin <i>lethal</i> long
Single cross-over.....	$W l m$ —red <i>lethal</i> miniature $w^* L M$ —eosin long $W L M$ —red long
Double cross-over.....	$w^* l m$ —eosin <i>lethal</i> miniature

TABLE 22
(Stock Lethal II)

	RED LONG ♀	WH. MIN. ♀	RED. MIN. ♀	WH. LONG ♀	RED LONG ♂	WH. MIN. ♂	RED MIN. ♂	WH. LONG ♂
A	428	381	161	125	0	362	31	21
B	365	337	168	175	1	276	45	60
C	509	502	229	232	0	368	65	74
D	316	329	95	115	0	305	26	63
E	318	289	104	88	1	278	31	62
F	312	259	95	81	0	259	19	61
G	73	105	24	27	0	94	3	26
H	124	104	68	52	1	105	14	36
I	118	114	50	46	1	117	16	33
J*	158	119	64	53	0	111	10	35
K	8	15	18	19	1	15	1	17
L	96	107	42	43	1	96	11	27
M	119	104	53	40	0	137	14	23
N	28	35	13	16	1	37	7	14
O	24	14	9	8	0	28	4	4
P	242	241	83	84	1	209	31	53
Q	271	302	114	116	1	338	31	63
	3509	3357	1388	1320	9	3135	359	672

* When Generation I was reached some of the red long daughters were bred to eosin miniature males (instead of using white miniature as before (A-I) under similar circumstances). Their offspring were as follows:

Red	long.	Wh. min.	Eos.	min.	Wh. long	Red	min.	Eos.	long
♀	♂	♂	♀	♂	♂	♀	♂	♀	♂
277	0	227	261	11	57	107	28	89	1

The long red females were again selected and bred to eosin miniature males to give generation J, K, L. Thus one generation is omitted in table 22, and from the summaries which include table 22.

Of the eight possible combinations, four carry lethal and will not be represented in the male classes of the next generation from the back-cross. The four surviving male classes represent:

- (1) A non-cross-over class (eosin miniature)
- (2) A single cross-over class (red miniature)
- (3) A single cross-over class (eosin long)
- (4) A double cross-over class (red long)

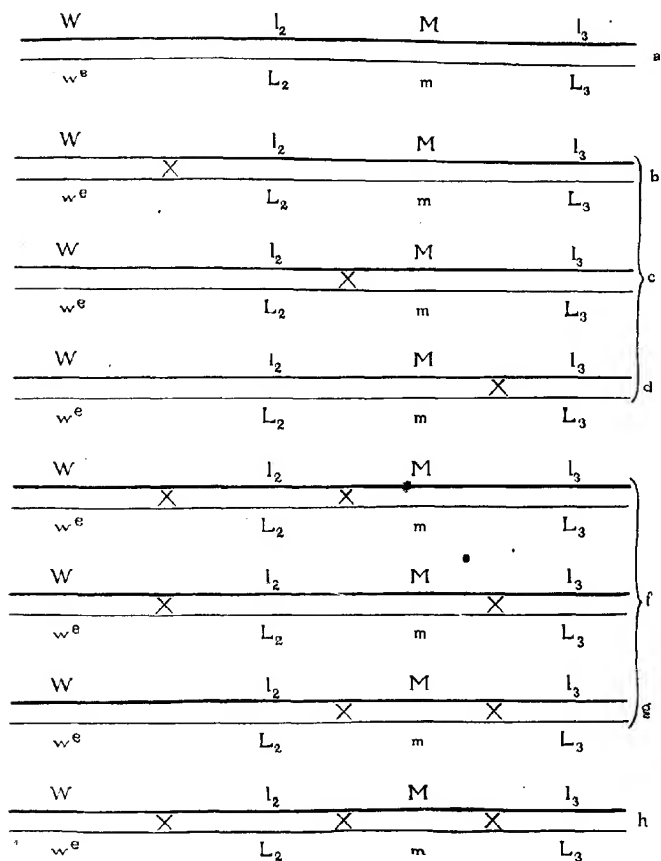


Figure F

TABLE 23

NO.	EOS. MIN.	RED MIN.	EOS. LONG	RED LONG	EOS. LETHAL	LETHAL MIN.	EOS. MIN.
XI	436	54	104	0	9.1	17.5	26.6
XII	290	48	45	0	12.5	11.7	24.2
XIII	394	51	47	0	10.4	9.6	20.0
XIV	455	69	56	0	15.2	12.3	27.5
XV ^a	77	21	31	3			
XV ^b	37	6	7	0			
XIX	220	45	77	2	13.7	22.4	35.5
XXII	3135	359	672	9	8.8	16.3	24.7
Total	4045	653	1039	14	9.9	15.6	25.1

In table 23 the data for the male classes available in the preceding tables are brought together in the first four columns. In the four columns that follow these, the percentages of crossing-over between: (1) eosin and lethal; (2) lethal and miniature; (3) eosin and miniature, are calculated for each of the experiments taken separately and for the sum total of the data.⁶

There is a considerable amount of difference in the percentages from the different experiments, as an examination of the table will show. If we take the totals as the more significant it appears that Lethal II lies nearly midway between eosin and miniature, but nearer to eosin. The total 'distance,' viz., 25 between eosin and miniature is smaller than that calculated from other data.

In the preceding experiments the females as well as the males give significant figures for eosin and miniature. In the next table, 24, these are brought together, and the percentage of crossing over between eosin and miniature calculated. This is 30.7 per cent.

In order to see whether the lethal factor has itself any influence on the results (which is not to be expected), the data from the sister individuals that gave a 1:1 ratio have also been utilized and are given in table 25. The percentage 'distance' is 32.4, which agrees with the calculation for the totals of the preceding table. Finally, the female classes alone are taken in the 2:1 count. These

⁶ The data in two cases, viz., 15a and 15b are not calculated separately, since the numbers are small.

give a percentage of 32.3. It seems to follow that the low percentages found when the males of the 2:1 ratio are taken alone is probably a disturbance due to viability in the males.

The linkage of Lethal II with eosin and with vermillion

As in the last case, the data for vermillion are to be considered. Figure C will serve to show the mode of entrance of the factors into the experiment if the factor V be used in place of M near

TABLE 24

NO.	SEX	RED LONG	WH. MIN.	RED MIN.	WH. LONG
XI	♀ 1:1	392	344	166	163
	♂ 1:1	245	332	159	132
	♀ 2:1	488	488	223	226
XII	♀ 2:1	367	327	164	159
XIII	♀ 2:1	467	410	230	162
XIV	♀ 2:1	479	450	227	229
XV*	♀ 2:1	113	104	58	59
XV ^b	♀ 2:1	50	46	14	16
XIX	♀ 1:1	313	282	142	140
	♂ 1:1	275	275	146	127
	♀ 2:1	283	230	130	131
XX*	♀ 1:1	1874	1612	1025	913
XXI	♀ 1:1	139	135	65	72
	♂ 1:1	112	120	66	44
XXII	♀ 2:1	3509	3357	1388	1320
Total		9106	8512	4103	3693

* The male class of XX is omitted because the white long males are far behind expectation.

TABLE 25

NO.	SEX	RED LONG	WH. MIN.	RED MIN.	WH. LONG
XI	♀	392	344	166	163
	♂	245	332	159	132
XIX	♀	313	282	142	140
	♂	275	275	146	127
XXI	♀	139	135	65	72
	♂	112	120	66	44
Total		1476	1488	744	678

which it really lies. The following list will serve to recall the possible combinations in the gametes of the F_1 female.

Non-cross-over.....	$\left\{ \begin{array}{l} W l_2 V \text{ —red lethal} \\ w^e L_2 v \text{ —eosin vermillion} \\ W L_2 v \text{ —vermillion} \\ w^e l_2 V \text{ —eosin lethal} \end{array} \right.$
Single cross-over.....	$\left\{ \begin{array}{l} W l_2 v \text{ —lethal vermillion} \\ w^e L_2 V \text{ —eosin} \end{array} \right.$
Double cross-overs	$\left\{ \begin{array}{l} W L_2 V \text{ —red} \\ w^e l_2 v \text{ —vermillion} \end{array} \right.$

In table 26 the available data are given in the first four columns, and the percentage distance between eosin and lethal, and lethal and vermillion, and eosin and vermillion in the following three columns. The calculations are given for the experiments taken singly, and for the totals. There is again a considerable discrepancy in the separate counts. The totals show that lethal lies between eosin and vermillion, but nearer to eosin. The total percentage distance between eosin and vermillion is 27.9.

As before, a calculation from the total data was made, table 27, on the basis of the male and female cases taken together, which

TABLE 26

NO.	EOS. VERM.	VERM.	EOSIN	RED	EOS. LETHAL	LETHAL VERM.	EOS. VERM.
XVI	464	48	109	5	8.5	18.2	25.1
XVII	219	33	68	0	10.3	21.2	31.5
XVIII	219	43	50	1	14.1	16.3	29.7
Total	902	124	227	6	10.4	18.5	27.9

TABLE 27

NO.	SEX	RED LONG	EOS. VERM.	VERM.	EOSIN
XVI	♀ 1:1	428	407	168	166
	♂ 1:1	321	420	120	78
	♀ 2:1	499	425	167	181
XVII	♀ 2:1	278	250	102	88
XVIII	♀ 2:1	264	211	107	84
Total		1790	1713	664	597

gives 26.5 per cent. Similarly the totals based on the experiments with 1:1 ratios gives 25.2; and those based on the females alone of the 2:1 ratios give 27.5. The difference in these is not greater than is expected. The lower percentage for the males taken alone is again probably due to viability.

Summary of linkage data of Part II

In the following table all the data available in the preceding pages (Lethal II) bearing on each linkage value have been brought together in a grand total:

	total	cross-overs	per cent cross-overs
White (or eosin) lethal II.....	8,152	812	10.0
Lethal II, miniature.....	6,407	974	15.2
Lethal II, vermilion.....	1,604	313	19.5
White (or eosin) miniature.....	36,022	11,048	30.7
Eosin (or white) vermilion.....	6,023	1,612	26.8

In the preceding table there are some discrepancies that appear when the percentages of crossing-over of the different counts of the same experiment are compared. Discrepancies like these may be due sometimes to insufficient numbers, sometimes to variations in differential viability (including the lagging behind of some of the male classes); in the case of lethals the absence of the 'contrary' classes (since one class dies)* makes it impossible to check up viability in the male classes. Special external and internal conditions may at times affect the degree of interchange between the homologous chromosomes, which, by changing the gametic ratio, will affect the realized classes.

* To determine the loci of factors accurately special studies and corrections are necessary. The present data pretend no more than to give the approximate positions of the loci, and, in this sense, the results are as consistent as is to be expected under the conditions of the experiments that were carried out with another end in view.

SEX-RATIOS HIGHER THAN 2:1

A glance at the last columns of the tables will show in many cases that the sex-ratio is higher than 2:1. Most of these differences are obviously chance deviations. There are some, however, that are so high as to suggest higher ratios. In fact, the start was made with a 5:1 ratio. It seemed at first as though those ratios higher than 2:1 might be due to two lethal factors that were simultaneously present, although this involves either two simultaneous mutations or the appearance of a new lethal in an egg that already contained one (through mutation or fertilization). Whether such an hypothesis is improbable will depend on how often lethal factors appear in stocks like these. Let us see how it might be shown that the high ratios could be explained as due to two lethals. If a third lethal l_3 were present it would be in all probability beyond miniature, or, like the second lethal, between miniature and white. On the first supposition the possibilities are shown in figure F. There are three single cross-over classes, three double cross-over classes and one triple cross-over. Of the sixteen possible classes of males only four would come through, as shown in the following list:

(a) Non-cross-over.....	$\left\{ \begin{array}{l} W \ l_2 \ M \ l_3 \text{ ---not realized} \\ w^e \ L_2 \ m \ l_3 \text{ ---cosin miniature} \end{array} \right.$
(b) Single cross-over.....	$\left\{ \begin{array}{l} W \ L_2 \ m \ l_3 \text{ ---red miniature} \\ w^e \ l_2 \ M \ l_3 \text{ ---not realized} \end{array} \right.$
(c) Single cross-over.....	$\left\{ \begin{array}{l} W \ l_2 \ m \ L_3 \text{ ---not realized} \\ w^e \ l_2 \ M \ l_3 \text{ ---not realized} \end{array} \right.$
(d) Single cross-over.....	$\left\{ \begin{array}{l} W \ l_2 \ M \ L_3 \text{ ---not realized} \\ w^e \ L_2 \ m \ l_3 \text{ ---not realized} \end{array} \right.$
(e) Double cross-over.....	$\left\{ \begin{array}{l} W \ L_2 \ M \ l_3 \text{ ---not realized} \\ w^e \ l_2 \ m \ L_3 \text{ ---not realized} \end{array} \right.$
(f) Double cross-over.....	$\left\{ \begin{array}{l} W \ L_2 \ m \ l_3 \text{ ---not realized} \\ w^e \ l_2 \ M \ L_3 \text{ ---not realized} \end{array} \right.$
(g) Double cross-over.....	$\left\{ \begin{array}{l} W \ l_2 \ m \ l_3 \text{ ---not realized} \\ w^e \ L_2 \ M \ L_3 \text{ ---cosin long} \end{array} \right.$
(h) Triple cross-over.....	$\left\{ \begin{array}{l} W \ L_2 \ M \ L_3 \text{ ---red long} \\ w^e \ l_2 \ m \ l_3 \text{ ---not realized} \end{array} \right.$

One of the four that are possible is the triple cross-over or red long. A triple cross-over is a very rare occurrence and would

not be expected within the numbers that appear in the experiment. The double cross-over is eosin long whose expectation is also rare. In table 11 there are two lots, Nos. 16 and 20, in which no males appear in this class, but as the expectation calls for very few flies here, it is unlikely that this can be the correct interpretation. If it were the correct interpretation we should get evidence of this third lethal when the red females of No. 16 were bred, as in table 12. But here there is no evidence of a third lethal, nor is there any in the following generations. It seems unlikely, therefore, that there can be any such lethal (i.e., one beyond miniature) involved in this experiment.

On the other hand, if the supposititious additional lethal should be between eosin and miniature, as shown in figure G, the effect would be to lower the class red miniature or eosin long below expectation for one lethal. In fact, the percentages of these classes are too low, but one would not be warranted, I think, from these data, in postulating a third lethal to account for this numerical discrepancy.

It seems probable, then, that the high ratios are either extreme cases of the 2:1 ratio, or that there is present some disturbing element not yet detected. The former view does not seem probable for the numbers are large. The second interpretation is more plausible, especially when the fact is recalled that the start was made from an exceptionally high ratio (5:1), and the line maintained by selecting the offspring of the pairs that showed the highest ratios.

There is another possibility that should at least be mentioned. The males hatch later than the females, and in the crowded bottles the males string out for some days after the females have ceased to hatch. In the present case there was some crowding, but the bottles were run to a finish, or practically so, in order to get the full male count. That the low male ratio is not due to this condition is shown by the fact that the non-cross-over class (eosin miniature males) compares favorably with the corresponding classes of females.

Sterility

An occasional test was made of the males that were obtained in the high sex-ratio pairs. Thus the sex-ratio from which table 1 came was 78♀♀ to 16♂♂. Every one of these sixteen males was sterile. (Of their sisters only 13 out of 78 were sterile, or at least gave no offspring).

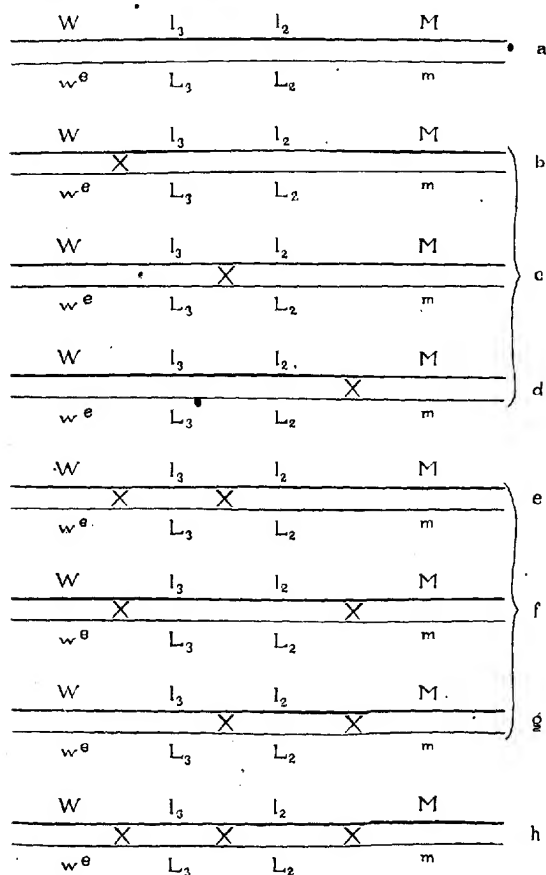


Figure G

In No. 39 (ratio of 115:33), seven out of fourteen females mated gave no offspring. One lot in table 1 shows a ratio of 116 ♀♀ to 46 ♂♂. Out of 31 ♀♀ tested, four produced nothing. Out of 14 ♂♂, seven were sterile.

One lot (xvii) of table 1 had a sex-ratio of 96 ♀♀ to 30 ♂♂. Out of thirty-nine females mated, only one gave no offspring. Six males tested were sterile.

No. 12 of table 1 had a sex-ratio of 122 ♀♀ to 32 ♂♂. Out of forty-three females mated, twelve produced nothing. Out of five males tested two produced nothing.

Summing up the results for the males, we get:

sex ratio	males tested	males sterile
78:16.....	16	16
116:46.....	14	7
96:30.....	6	6
122:30.....	5	2
Total.....	41	31

AN HYPOTHESIS TO ACCOUNT FOR THE EXCEPTIONALLY HIGH RATIOS

The following very high sex-ratios have been recorded for *Drosophila*:

	females	males
1	0	135
2	108	0
3	104	0
4	73	0
5	63	0
6	40	0
7	43	0
8	33	0
9	31	0
1	68	0
2	52	1
3	30	0

¹ Quackenbush, L. S. Unusual broods of *Drosophila*. Science, N.S., vol. 32, p. 183, August 5, 1910.

	females	males	ratio
	312	3	104:1
	169	5	34:1
Rawls ⁸	276	26	10:1
	291	54	5:1

In those cases where the offspring were tested (Rawls) it was found that the very high ratio disappeared. It seems to be due to a particular combination that was subsequently lost. If we assume that two linked lethals occurred, one in each of the sex chromosomes of the mothers that gave these high ratios, an explanation of the results is apparent. Such a female herself could live, since the lethals affecting different parts of the individual have each its normal allelomorph in the other sex chromosome. The eggs produced would be in the main of two kinds—half containing one lethal, half the other. Since all of the sons derive their single sex-chromosome from the mother, they will perish. But if crossing over in some of the eggs of the F_1 female should occur then in such eggs one of the chromosomes will get both lethals, and the other both the normal allelomorphs. The former type of egg, if fertilized by a male-producing sperm, would fail to produce a male; the latter type of egg would produce a normal male. The few males that appear may represent this class.

There are two ways in which the original double lethal female might be imagined to arise. A new sex-linked lethal might appear in one of the sperm-cells. If such a spermatozoon fertilizes an egg already containing a lethal a double lethal female that is viable would result. The frequency with which lethal mutants have turned up would make plausible this assumption. The same end would be reached if, in a female already containing one sex-linked lethal, another lethal should appear in the other sex-chromosome.

For two other results no hypothesis even can be suggested. First, the remarkable sterility found in the F_1 males when these high sex-ratios appeared; second, the converse case described by Quackenbush where 135 males and no females appeared.

⁸ Rawls, E. Sex ratios in *Drosophila ampelophila*. Biol. Bull., vol. 24, January, 1913.

I wish to acknowledge my indebtedness to Mr. A. H. Sturtevant and Mr. C. B. Bridges with whom I have discussed the theoretical questions involved in the experiments, and also my indebtedness to Miss E. M. Wallace and to Mr. J. S. Dexter who have made the greater part of the 55,000 records that furnish the evidence on which the conclusions rest.

CLUSTER FORMATION OF SPERMATOOA CAUSED BY SPECIFIC SUBSTANCES FROM EGGS

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INTRODUCTION

In several papers F. Lillie¹ has described a very interesting specific phenomenon of apparent sperm agglutination which occurs when the sperm is mixed with sea water which has been in contact for a short time with a sufficient quantity of eggs of the same species (*Arbacia* and *Nereis*):

In the case of *Arbacia* the addition of two or three drops of egg-sea water $\frac{1}{4}$ (i.e., one volume of eggs to four volumes of sea water) which has stood half an hour, to about 2 cc. of fresh milky sperm suspension causes formation of agglutinations 1 to 2 mm. in diameter in a few seconds. The agglutination may be so strong that the fluid between the white agglutinated masses appears perfectly clear. The masses gradually fade from view in a few minutes, but microscopic agglutinations may remain half an hour or more.

The agglutination is, therefore, only transitory or reversible, as Lillie states. It is specific since e.g., the supernatant sea water of *Arbacia* eggs acts only on *Arbacia* sperm and not on other sperm.

It is very natural that Lillie should have been led to the idea that such a striking specific phenomenon as this agglutination must play a rôle in the process of fertilization and he has recently offered a very carefully worked out hypothesis which makes this phenomenon of agglutination not only the center of the process of fertilization and of artificial parthenogenesis but he even hints that it may be involved in the phenomena of heredity.

¹ Science, N.S., vol. 36, p. 527, 1912; Journ. Exper. Zool., vol. 14, p. 515, 1913.

Lillie's theory of the phenomenon of agglutination is an application of Ehrlich's side-chain theory, a fact which gives it additional interest.

In previous papers I have described the secretion of a substance by the ova of the sea urchin, *Arbacia*, in sea water, which causes agglutination of the sperm of the same species. The eggs of *Nereis* also secrete a substance having a similar effect upon its sperm. I therefore named these substances sperm-isoagglutinins. During the present summer I have ascertained that in the case of *Arbacia*, and presumably also of *Nereis*, the agglutinating substance is a necessary link in the fertilization process and that it acts in the manner of an amboceptor, having one side-chain for certain receptors in the sperm and another for certain receptors in the egg. As this substance represents, presumably, a new class of substances, analogous in some respects to cytolytins, and as the term agglutinin defines only its action on sperm suspensions, I have decided to name it fertilizin.²

The writer had for many years observed that when the eggs of the Californian sea urchin *Strongylocentrotus purpuratus*, were fertilized with sperm of their own species the spermatozoa would not always scatter but would form small clusters which were often visible with the naked eye. These clusters would disappear in a few minutes. The whole phenomenon resembles strikingly the phenomenon described by Lillie under the name of sperm agglutination, and is possibly identical with it.

The writer was interested to find out what the conditions of this cluster formation of the sperm and its relation to the process of fertilization were. Since he is not certain whether this cluster formation observed by him on the Californian sea urchin is identical with the observations of Lillie on the agglutination of sperm in *Arbacia*, he will confine himself to a discussion of his own experiments and observations, leaving it for future work to decide to what extent they harmonize with Lillie's observations and conclusions.

² Science, N.S., vol. 38, no. 980, p. 524, October 10, 1913.

METHOD OF OBSERVATION AND THE SPECIFIC CHARACTER
OF CLUSTER FORMATION

If we put one or more drops of a very thick sperm suspension of the Californian sea urchin, *S. purpuratus*, carefully into the center of a dish containing 3 cc. of ordinary sea water, and let the drops stand for one-half to one minute, and then by gentle agitation mix the sperm with the sea water, the at first rather viscous mass of thick sperm is in a few seconds distributed equally in sea water and the result is a homogeneous sperm suspension.

When the same experiment is made with the sea water which has been standing for a short time in a dish over a large mass of eggs of the same species, the result is entirely different. The thick drop of sperm seems to be less miscible and instead of a homogeneous suspension of sperm we get as a result the formation of a large number of distinct clusters which are visible to the naked eye and may possess a diameter of 1 or even 2 mm. The rest of the sea water is almost free from sperm. These clusters of spermatozoa last for from two to ten minutes and then dissolve by the gradual detachment of the spermatozoa from the periphery of the clusters. This phenomenon is to some extent specific. The sperm of the sea urchin *Strongylocentrotus purpuratus*, will give the cluster formation with the supernatant sea water of the eggs of *S. purpuratus*; the sperm of the sea urchin *S. franciscanus* will give the cluster formation with the supernatant sea water of eggs of its own kind as well as with the supernatant sea water of the eggs of *S. purpuratus*. In the latter case the clusters dissolve a little more quickly than if franciscanus sperm is added to the supernatant sea water of franciscanus eggs. The sperm of *purpuratus* will not form clusters with the supernatant sea water of the eggs of franciscanus. It is of interest that the specificity is not reciprocal in the case of these two sea urchins.

The sperm of neither formed clusters with the supernatant water of starfish eggs or of mollusc eggs.

The sperm of starfish (*Asterias ochracea* and *Asterina*) gave no cluster formation with the supernatant sea water of their own eggs or of the eggs of the two sea urchins.

We shall have to return to these data in a later chapter when we discuss the relation between cluster formation and fertilization.

The following experiments were carried on with the sperm of *S. purpuratus* and the supernatant sea water of the eggs of the same species, unless the contrary is stated.

APPARENT SURFACE TENSION PHENOMENA AND CLUSTER FORMATION

In analyzing the formation of these clusters the writer was struck with the fact that the cluster formation showed peculiarities which resembled the action of surface tension. The clusters were usually spherical, or had a tendency to become so. When two clusters were brought into contact with each other they fused at once into one spherical cluster with a larger radius, a behavior which would also be observed in the case of drops of substances immiscible with water under similar conditions. The formation of the clusters themselves resembled surface tension phenomena. When a drop of *purpuratus* sperm is gently agitated in a little dish with a few cubic centimeters of ordinary sea water streaks and cylindrical masses of sperm are formed in the water which, however, show nothing that reminds one of surface tension phenomena. The spermatozoa are gradually scattered without surface tension offering any resistance to the scattering.

If the same experiment is made in the supernatant sea water from the eggs—in egg-sea water—the streaks of sperm produced by agitation behave somewhat like cylinders of a very viscous substance which is immiscible with water, e.g., a viscous oil or a calcium soap. Short streaks or cylinders contract into spherical masses, the above described clusters; and long cylinders break up into a series of small clusters.

In an attempt to account for this apparent or real rôle of surface tension in cluster formation the writer thought first of the possibility that it might be due to an agglutination of the masses of sperm under the influence of the egg-sea water. A study of the real phenomenon of sperm agglutination, however, showed that it does not lead to any formation of spherical clusters. The writer had shown eleven years ago that real sperm agglutination

can be produced if we add 2 or 3 cc. of $\frac{N}{10}$ NaOH to 50 cc. of sea water.³ He found recently a good method of producing sperm agglutination with less alkali in the case of the sperm of starfish. When this sperm is put into 50 cc. sea water + 0.5 cc. $\frac{N}{10}$ NaOH it shows a tendency to agglutinate only after about one hour. But we can produce a real agglutination of the spermatozoa after about only twenty minutes when we put the sperm into the supernatant sea water of eggs or sperm of *purpuratus*. This agglutination is not specific, since it can also be produced by a great many other substances, e.g., cattle serum or even white of egg. In this case the spermatozoa at first stick together to form short rows or threads; and later the threads begin to stick together and form irregular networks. At no time is there any appearance of cluster formation or anything suggesting the phenomena of surface tension.

The writer is therefore under the impression that the cluster formation of the sperm in the supernatant sea water of its own eggs is a phenomenon of a different type from agglutination.

MOTILITY OF SPERM AND CLUSTER FORMATION

In observing the clusters the writer was struck with the fact that the spermatozoa at the periphery of a cluster are in free progressive motion, a fact which is incompatible with the assumption of agglutination. When the clusters were small or when the sperm suspension was thin it was possible to observe the spermatozoa which are in the center of the cluster. It was seen that the spermatozoa in the center also were in very lively motion, with the possible exception of small lumps or groups of spermatozoa which may have stuck together. The clusters reminded the writer of a dense swarm of insects which move like a coherent mass through space. These clusters move like one solid body through the water, notwithstanding the fact that the individual spermatozoa are free to scatter.

Under the influence of these observations the writer formed the idea that the cluster formation and possibly the apparent phenomena of surface tension might be the outcome of some tropistic

³ Loeb, Arch. f. d. ges. Physiol., Bd. 99, p. 323, 1903; Bd. 104, p. 325, 1904.

reaction of the spermatozoa. If this were the case, we should expect that anything that diminished the motility of the spermatozoa would lessen the tendency of the sperm to form clusters, and if the sperm were paralyzed completely the cluster formation would also cease completely.

It was easy to show that both assumptions were correct. To 3 cc. of a dense sperm suspension in ordinary sea water were added 1 or 2 drops of a 0.1 per cent solution of NaCN, and the whole thoroughly mixed. In one or two minutes the sperm lost its motility and did not regain it when put into sea water. When one or several drops of this immobilized sperm were added to the egg-sea water and when after one minute the dish was gently agitated, the sperm behaved exactly as if it had been put into normal sea water. Not a trace of cluster formation was noticeable; a slight agitation sufficed to bring about a perfectly homogeneous mixture of the sperm in sea water. After two hours the sperm became motile again when put into sea water. When such sperm, after the recovery of its motility, was put into egg-sea water a very powerful cluster formation occurred again.

These experiments were varied and always proved definitely that the whole phenomenon of cluster formation existed only when the sperm was motile.

There are other ways of paralyzing the spermatozoa. When the sperm of *purpuratus* is heated to a temperature of 35°C. or even 36°C. the sperm remains motile and the phenomenon of cluster formation is striking when a drop of such sperm is added to 3 cc. of egg-sea water and the mass is agitated. As soon as sperm is brought to a temperature of 37.6° or above and rapidly cooled, the motility is gone and no cluster formation takes place.

The same experiment was made with the sperm of *Strongylocentrotus franciscanus* and the supernatant sea water of eggs of the same species. When the sperm is heated to a temperature of 36.2° its motility continues and the cluster formation is not diminished. When the sperm is heated for one minute to a temperature of 37° the motility of the sperm is only diminished and only small clusters are formed. If the sperm is heated to 38° the motility of the sperm disappears and the phenomenon of cluster formation is impossible.

The same result is obtained if the motility of the sperm is diminished or annihilated through the addition of KCl to sea water.

These are all very striking demonstration experiments, which leave no doubt that the cluster formation and the apparent surface tension phenomena depend exclusively on the motility of the spermatozoa.

On the other hand, the writer convinced himself that in the true phenomena of sperm agglutination, the motility of the sperm is of no concern. We have mentioned the fact that the sperm of *Asterias*, when it has been in 50 cc. sea water + 0.5 cc. $\frac{N}{10}$ NaOH for fifteen or twenty minutes, undergoes a real agglutination when mixed with the supernatant sea water of different kind of eggs or of cattle serum. This real agglutination takes place just as well after the spermatozoa have been completely immobilized by KCN as before. We may therefore be sure that the cluster formation is not due to an agglutination.

CLUSTER FORMATION A POSSIBLE TROPIC REACTION

The writer's idea of a tropism underlying the cluster formation is at present only a mere working hypothesis about which it is therefore not necessary to say much. It is briefly this, that the spermatozoa which are rendered extremely active by the egg-sea water are at the same time repelled by it, in other words, that they possess a negative chemotropism or a negative differential sensibility towards the egg-sea water; while any small or large mass of spermatozoa at the boundary of or in egg-sea water acts as a center to which the isolated spermatozoa are positive. This would account for the fact that the cluster formation is a function of the motility of the spermatozoa and could also account for the apparent surface tension phenomena. We should also understand why the cluster formation (just like Lillie's 'agglutination') lasts only a few minutes. Since the egg-sea water must gradually diffuse into the mass of spermatozoa, the boundary at which they are repelled must finally cease to exist. As soon as the concentration of the active substance of the egg-sea water is the same or almost the same in the cluster or the mass of spermatozoa as in the surrounding sea water there is no more force active which may induce or preserve the cluster formation.

The idea of a negative reaction of the spermatozoa to the egg-sea water is in contradiction to Lillie's statement that the spermatozoa are positive to the egg-sea water. The writer is not quite sure whether Lillie's statement is based on a correct interpretation of his observations.

Lillie introduced a drop of *Arbacia* egg-sea water into a suspension of *Arbacia* sperm under a cover glass. In this case a dense ring of spermatozoa was formed "at the margin of the drop with a simultaneous formation of a clear external zone about 1.5 to 2 mm. wide; the ring then breaks up into small agglutinated masses and so becomes beaded" (p. 550). In the interior of the drop very few spermatozoa are found. If the spermatozoa were positively chemotropic to the egg-sea water, as Lillie suggests, they should rush into the drop instead of forming a ring around it. The writer is inclined to interpret this formation of a ring with a clear external zone around it as an indication that the spermatozoa are negatively chemotropic to the strong egg-sea water and possibly positively chemotropic to the more diluted egg-sea water or to the dense collection of spermatozoa in the ring. Those in touch with the margin of the drop are repelled by the drop and those at some distance from the drop are attracted towards the ring or towards the drop. This creates the dense ring next to the drop of egg-sea water and explains the formation of the clear space externally to the ring.

When a cluster scatters it does not scatter equally but one notices that isolated microscopic lumps or beads of spermatozoa may be left in the center of the original cluster. Later these beads or lumps scatter also. It is possible that the spermatozoa constituting these lumps or beads stick temporarily together and that this is caused by a specific substance contained in the egg-sea water. This agglutination, however, cannot account for the fact that cluster formation is only possible if the sperm is very motile. The cluster formation is, aside from the increased motility, the only striking phenomenon which the sperm of the Californian sea urchin shows in the presence of egg-sea water.

The writer wishes the statements of this paragraph to be taken only provisionally.

THE CONDITIONS WHICH DETERMINE THE SCATTERING OF
THE CLUSTER

The clusters (just like Lillie's 'agglutinations') have only a short duration of from two to ten minutes, as the circumstances may be. It was of interest to find out some of the conditions which determine their duration. It was found that the stability of the clusters depends upon the alkalinity of the sea water. In a neutral solution the big clusters may last a considerable time, half an hour or more, while in sea water to which a sufficient amount of alkali has been added the clusters may scatter in a minute. The reader must remember that if we add HCl or NaOH to sea water part of the added acid or base will be neutralized by the carbonates and phosphates of the sea water.

To 5 cc. supernatant sea water from *purpuratus* eggs were added 0, 1, 2, 3, 4 drops of $\frac{N}{10}$ NaOH and 3 drops of a dense suspension of *purpuratus* sperm were added to each. In all dishes a large cluster was formed. In the dishes with 4 and 3 drops of NaOH the clusters were dissolved almost instantly after formation, in the dish with 2 drops the resolution occurred more slowly and it lasted longest—about eight minutes—in the sea water to which no alkali was added.

In a second experiment to 5 cc. of the same egg-sea water 0, 1, 2, 3 and 4 drops of $\frac{N}{10}$ HCl were added, and then *purpuratus* sperm introduced. In 5 cc. egg-sea water + 4 drops of HCl no cluster formation occurred, probably because the motility of the sperm was too rapidly annihilated. In the dish with 3 drops HCl only a trace of cluster formation was noticeable; with 2 drops a moderate cluster formation occurred and only in the two dishes with 0 and 1 drop of HCl was the cluster formation very powerful, since here the motility of the spermatozoa was not impaired. In the egg-sea water without acid the clusters disappeared much more quickly than in the sea water with 1 or 2 drops of acid.

Experiments in which neutral artificial sea water was substituted for normal sea water showed that at the point of neutrality the cluster formation is most durable. The big clusters continued to exist as long as half an hour, while in alkaline solutions they disappeared very rapidly. In acid solutions no cluster formation

was possible probably on account of the fact that the spermatozoa became immobile.

These and other experiments prove that an increase in the alkalinity of the solution shortens the duration of the clusters; in spite of the fact that an increase in alkalinity of the sea water favors the real agglutination of sperm.

The writer tried then to ascertain which salt solutions favor the formation of these clusters. To investigate this point the ovaries and testes of *purpuratus* were washed in an $m/2$ NaCl solution and then put directly into another $m/2$ NaCl solution without coming in contact with sea water. It was found that the supernatant solution of the eggs did never, or only exceptionally, give rise to cluster formation with the NaCl sea water; the reason for this may be partly the fact that the spermatozoa are practically inactive in a pure NaCl solution and that although the presence of the supernatant NaCl solution from the eggs stimulates the spermatozoa into activity this may not always be sufficient. The addition of KCl does not materially improve the cluster formation, the addition of the chlorides of Mg, Ca, Sr and Ba and of $MgSO_4$ vastly increases the cluster formation or induces it in an otherwise inefficient NaCl solution.

It is not possible to draw any conclusions from these facts upon the nature of the process underlying it.

THE ORIGIN OF THE SUBSTANCE CAUSING THE CLUSTER FORMATION

Lillie assumes that the substance which causes the phenomenon described by him as agglutination is given off by the egg itself though he states that the jelly which surrounds the egg—viz., the chorion—is saturated with this substance. The writer was curious to know whether the phenomenon of cluster formation depends upon a substance given off by the egg or whether it is due to a substance originating from the chorion. It could easily be shown that the latter is the case. Herbst had stated that the chorion of the sea urchin egg can be dissolved by acid. The writer therefore put a mass of eggs of *purpuratus* for three minutes

into 50 cc. sea water + 3 cc. $\frac{N}{10}$ HCl. The eggs were constantly squirted with a pipette to prevent them from sticking to the glass and were then transferred to normal sea water. They were then washed five times in succession in normal sea water under constant squirting with a pipette and then left standing in a refrigerator with a small volume of sea water. At no time did the sea water in which these eggs were kept give any trace of a cluster formation with fresh sperm. The supernatant sea water was tested a few hours after the acid treatment and two or three times daily on four consecutive days. These eggs which had apparently lost their chorion had permanently lost the power of giving off to the sea water a substance which causes the cluster formation of the spermatozoa of the same species. If the substance were constantly given off by the egg it should have been found after some time in the supernatant sea water. The experiment was repeated a number of times with the same negative result.

On the other hand, it was easy to show that the acid sea water (50 cc. sea water + 3 cc. $\frac{N}{10}$ HCl) in which the eggs had been washed contained the substance which is responsible for the cluster formation in large quantities. This acid sea water was filtered and the filtrate neutralized with NaOH (with neutral red as an indicator). The neutralized sea water gave with sperm of the same species a very powerful cluster formation.

This neutralized sea water kept the power of inducing cluster formation for about three days (during which time it stood in the refrigerator) but had lost it the fourth day.

This seems to indicate that the substance causing cluster formation is derived from the jelly-like substance surrounding the egg (the chorion) but does not emanate from the egg itself.

If this substance which causes the cluster formation should be identical with the substance which Lillie calls 'fertilizin,' which is very probable, it is obvious that his conclusion that the substance comes from the egg is untenable. This would also make it impossible to attribute to this substance a rôle in the process of artificial parthenogenesis.

CLUSTER FORMATION AND FERTILIZATION

Lillie's application of the side-chain theory to the problem of fertilization rests on the assumption that the substance which causes the phenomenon he describes as agglutination is indispensable for fertilization. This 'fertilizin' is in his theory an amboceptor which must combine at one end with the spermatozoön at another end with the egg; the 'fertilizin' when in combination with the spermatozoön undergoes a change and then fertilizes the egg. It is therefore a substance given off by the egg which in Lillie's opinion causes its fertilization, and not, as we all had hitherto assumed, one or more substances contained in the spermatozoön.

We have just seen that the substance which causes the cluster formation does not come from the egg but is given off by the chorion—or possibly is the chorion itself which is slowly soluble in sea water.

We can show in a number of different ways that eggs which have lost or do not possess the power of giving off a substance which induces cluster formation may possess the normal power of being fertilized. If we treat eggs of *purpuratus* for three minutes in 50 cc. sea water + 3 cc. $\frac{N}{10}$ HCl and wash them about five times in sea water they have lost the power of causing the cluster formation of the sperm of *purpuratus*. Such eggs can be fertilized immediately after the washing or at any time during the next two or three days if they are kept in the refrigerator. Their power of being fertilized is not in the least impaired. One hundred per cent of the eggs were invariably fertilized and the fertilization took place instantly after the addition of sperm. Practically all the eggs developed. The membrane was slightly abnormal which was an after-effect of the acid treatment. The power of the eggs of being fertilized remains unimpaired while their power of giving off substances which cause cluster formation is completely and permanently lost.

When we treat the eggs with a fatty acid instead of with a mineral acid they form, when transferred to normal sea water, a fertilization membrane. The fatty acid also dissolves the chorion and such eggs when washed afterwards lose their power of induc-

ing cluster formation of sperm. It is interesting that Lillie states that such eggs lost also their power of causing agglutination, which seems to suggest that Lillie's 'agglutination' and the writer's 'cluster formation' may be the same thing. Lillie states that such eggs which have formed a fertilization membrane have lost their power of being fertilized and he sees in this a support of his theory that without 'fertilizin' the egg can no longer be fertilized.

Eggs which have formed a fertilization membrane under the influence of butyric acid can easily be fertilized with sperm if the membrane is torn by shaking. The writer has repeated this experiment this winter and confirmed the earlier observations by Kupelwieser and himself to the same effect. He also made sure that the eggs which after the artificial membrane formation can be fertilized with sperm have completely lost the power of causing cluster formation.

We thus see that complete loss of the power of inducing cluster formation can be combined with maximal power of the eggs of being fertilized.

HYBRIDIZATION AND CLUSTER FORMATION

The best test for a possible connection between fertilization and cluster formation is afforded in the phenomena of hybridization. If the phenomenon of cluster formation were inseparably associated with the power of the eggs of being fertilized, we should expect that sperm should only be able to fertilize the eggs of a species if the egg-sea water of the same species caused the cluster formation of the sperm.

It is easy to show that no connection of this type exists. It is impossible to call forth cluster formation of the spermatozoa of the starfish *Asterias ochracea* with the egg-sea water of *purpuratus* and yet 100 per cent of the eggs of *purpuratus* can be fertilized with the sperm of *ochracea* and as many as 80 per cent of these eggs may develop. The writer showed that this hybridization takes place only in hyper-alkaline sea water and it was therefore necessary to test the possibility of cluster formation in both neutral and alkaline sea water; these tests gave always absolutely negative results.

The sperm of *purpuratus* shows no trace of cluster formation with the egg-sea water of *franciscanus* and yet the eggs of *franciscanus* are readily fertilized with the sperm of *purpuratus*.

If the cluster formation were caused by a substance which was necessary for fertilization in the sense of Lillie's theory these and probably many other hybridizations which occur should be impossible.

It often happens that in hybridization less than 100 per cent of the eggs are fertilized. The writer tried whether the yield of fertilized eggs could be increased if the egg-sea water from the species which furnishes the sperm be added to the mixture. This would furnish the sperm with the specific 'amboceptor.' It was found that hybridizations occur just as well if not better in normal sea water than if the egg-sea water from the species from which the sperm is taken be added.

All these facts contradict the assumption that the substance which induces the cluster formation of the spermatozoön is necessary for fertilization.

ARTIFICIAL PARTHENOGENESIS AND CLUSTER FORMATION

The writer has expressed the idea that the causation of the development of the egg either by a spermatozoön or by the agencies of artificial parthenogenesis is due to an alteration of the cortical layer of the egg which may or may not be accompanied by a membrane formation; and he has shown that all hemolytic substances are able to bring about this alteration. Lillie agrees with this idea but differs in regard to the origin of the agent which causes this change in the cortical layer of the egg in the case of fertilization by a spermatozoön. According to the writer, this change is caused by a substance contained in the spermatozoön while Lillie assumes that it is a substance contained in the egg which must, however, be activated by the spermatozoön in order to cause the alteration of the surface of the egg. It seems to the writer that Lillie's assumption is unnecessarily complicated. Moreover, if it should turn out that the substance which is responsible for the cluster formation is identical with the substance which Lillie calls 'fertilizin'—which is very likely the case—

Lillie's theory becomes untenable, since this substance does not, in all probability, originate from the egg but from the chorion and since there is, as we have seen, no connection between the presence of this substance and the power of the eggs of being fertilized.

A second difficulty which Lillie has not considered lies in the fact, that the writer has shown that in addition to the membrane forming substance still another, namely a corrective agency, is necessary for the causation of the development of the egg. The cortical change induces development but the egg as a rule perishes if the second factor of artificial parthenogenesis is not applied (hypertonic solution or lack of oxygen). The writer is suspicious that even a third factor may be implied. It is under these circumstances difficult to see how the assumption that the 'fertilizin' causes development—leaving aside all other objections—can act as an adequate substitute for the known facts of artificial parthenogenesis.

Lillie sees a proof for his idea in the statement of Glaser⁴ that the filtrate from eggs of *Arbacia* ground up with an equal volume of sea water will cause normal unfertilized eggs of the same species to undergo one or more cell divisions if they are transferred from the filtrate after one or two hours to normal sea water. Lillie concludes from this that the egg contains its own fertilizing substance, the 'fertilizin,' and that it was this 'fertilizin,' liberated from the eggs when they were ground up and contained in the filtrate, which induced the cell division of the *Arbacia* eggs in Glaser's experiments. Waiving the question of how this 'fertilizin' was 'activated' without the presence of sperm, the writer sees no reason to assume that the egg extract acted through the 'fertilizin,' or any other specific substance, since he has shown that a large number of non-specific substances are able to induce the first cell divisions (without membrane formation) in the egg of *Arbacia*, e.g., traces of any weak base like HN_3 , or protamine.⁵ A slight increase of osmotic pressure could also have such an effect. It even suffices to let the eggs of certain females lie for

⁴ Science, N.S., vol. 33, no. 973, p. 446, 1913.

⁵ Jour. Exp. Zool., vol. 13, p. 577, 1912; Arch. f. Entw. u. Vererb., Bd. 38, p. 409, 1914.

some time in sea water.⁶ The writer fails to see any reason for assuming that the cell division in Glaser's experiments was induced by 'fertilizin.' The fact that the composition of the mixture which he used was unknown is not sufficient proof that 'fertilizin' was the active agency.

In conclusion, the writer would like to call attention to the fact that the cluster formation described in this paper or the agglutination of sperm described by Lillie inhibit the fertilizing effect of the spermatozoa instead of enhancing it, since the cluster formation prevents the spermatozoa from reaching the egg. Even from a teleological viewpoint it is difficult to understand why a substance which only prevents the fertilizing action of the sperm should be a necessary link in such action. The writer is inclined to believe that the cluster formation or agglutination of sperm does not occur when fertilization takes place under natural conditions.

The writer is not quite sure whether his interpretation of the cluster formation of the spermatozoa as a tropistic reaction will have to be modified or not. If it should turn out to be essentially or partly correct, it might be worth while to point out that we have many examples of tropistic reactions which are of no possible use to the species, e.g., all the phenomena of galvanotropism which are only laboratory products.

The writer takes pleasure in expressing his thanks to Prof. S. S. Maxwell of the University of California for his kindness in putting the Herzstein Laboratory at New Monterey at the writer's disposal.

SUMMARY

1. The writer describes the formation of clusters of spermatozoa which is observed when the sperm of a sea urchin is put into the supernatant sea water of eggs of the same species. This specific phenomenon of cluster formation may possibly be identical with the specific phenomenon described under the name of sperm agglutination in recent publications of F. Lillie.

⁶ Arch. f. Entwicklungsmechn. d. Organ., Bd. 36, p. 626, 1913.

2. The cluster formation resembles the phenomena of surface tension in various respects, e.g., inasmuch as the clusters are spherical or tend to assume a spherical shape, and inasmuch as the fusion of two clusters results in the formation of a larger spherical cluster. When sperm is put into ordinary sea water or the supernatant water of foreign eggs these apparent surface tension phenomena are not observed. In real sperm agglutination neither cluster formation nor the above-mentioned surface tension phenomena are noticeable.

3. It was found that the cluster formation is a direct function of the motility of the spermatozoa. As soon as the spermatozoa are immobilized by NaCN or by high temperature or by KCl the cluster formation ceases; as soon as the motility of the spermatozoa returns the cluster formation occurs again when the sperm is put into the supernatant sea water of eggs of the same species. The real agglutination of sperm occurs just as well when the sperm is immobilized as when it is motile. The cluster formation is therefore not a form of agglutination.

4. The clusters last only a few minutes, like Lillie's sperm 'agglutinations.' The writer has found that in a neutral solution they last much longer than in an alkaline solution and that they scatter the more rapidly the more the alkalinity of the sea water is raised by the addition of NaOH.

5. The writer offers tentatively a working hypothesis which assumes that the phenomenon of cluster formation is essentially or partly due to a negative chemotropism of the spermatozoa to the egg-sea water.

6. It is shown that eggs which have been treated with acid sea water lose permanently their power of producing a substance which causes the cluster formation of the spermatozoa of their own species; while the acid sea water in which the eggs were treated when filtered and neutralized with NaOH induces a very powerful cluster formation. If it is true that the acid dissolves the chorion (the jelly-like substance surrounding the egg) this experiment would prove that the substance which causes the cluster formation is not formed in the egg but in the chorion. If this substance is identical with the substance which Lillie calls

'fertilizin,' his theory concerning the rôle which this substance plays in the fertilization and development of the egg will meet with serious difficulties.

7. It is shown that eggs which have been treated with a mineral acid like HCl and which have permanently lost the power of causing a cluster formation of the spermatozoa can nevertheless all be fertilized with sperm of the same species and that the rapidity with which the sperm fertilizes these eggs is equal to that with which normal eggs are fertilized. When the acid used was a fatty acid and when membrane formation occurred the eggs also lost permanently their power of inducing cluster formation but retained their power of being fertilized by sperm, provided that the membrane was first torn.

8. The supernatant sea water of the eggs of *Strongylocentrotus franciscanus* will not induce cluster formation of the sperm of *Strongylocentrotus purpuratus*; yet the latter sperm fertilizes the eggs of *franciscanus*. The sperm of *Asterias ochracea* undergoes no cluster formation in the supernatant sea water of *Strongylocentrotus purpuratus*, no matter whether the sea water is normal or hyperalkaline although the starfish sperm readily fertilizes most or all the eggs of *Strongylocentrotus purpuratus* in hyperalkaline sea water.

9. The facts mentioned under paragraphs 7 and 8 prove that the substance which is responsible for the cluster formation is not necessary for the process of fertilization.

FERTILITY AND STERILITY IN DROSOPHILA AMPELOPHILA

I. STERILITY IN DROSOPHILA WITH ESPECIAL REFERENCE TO A DEFECT IN THE FEMALE AND ITS BEHAVIOR IN HEREDITY

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INTRODUCTION

The following series of papers embodies the results of my work on "Fertility and Sterility in *Drosophila ampelophila*." The first paper deals primarily with a case of sterility in which the female is affected, together with the effects of selection upon female sterility and its behavior in transmission. The second paper is concerned with the low fertility of a mutant, and the behavior of this low fertility in heredity. The third study deals with the effect on fertility of crossing different races. The fourth study treats of the effect on fertility of crossing within and without an inconstant race. The work has been in continual progress since the fall of 1911 when it was undertaken at the suggestion of Prof. T. H. Morgan.

Two investigators have studied sterility in *Drosophila*. Castle and his students found sterile individuals of both sexes in their

cultures. Moenkhaus found in his strains that sterility was practically confined to the males. He attempted to harmonize Castle's results with his own by the assumption that each had used a different measure of productiveness. Both investigators are in agreement that inbreeding can not be the cause of sterility, and that sterility is amenable to selection. From Castle's paper I gather that he considers low productivity and complete sterility in the female as related and that sterility in this case is related to egg structure. Moenkhaus seems to imply that sterility is due to some condition of the sperm. Evidence that bears on these questions is given in the following pages.

I wish to state in the beginning that my results warrant me in making a sharp distinction between fertility and sterility as it actually exists in the strains that I have used. Fertility, as I have found it, does not grade into complete sterility. There may however be gradations in fertility. The total sterility that appears in my cultures bears no relation to low fertility. Sterility as it affects the flies in my strains is due to a different condition from that operating to reduce the fertility. This statement is certainly true of the sterility as it affects the female. The distinction paves the way for bringing the results of other investigators under a common point of view. Failure to distinguish between sterility and fertility has I suspect led to confusion, certainly in the work of Castle, and probably in the results of Moenkhaus, because as I shall show the two things may relate to quite different phenomena. The inheritance of sterility and fertility in *Drosophila* must be separately treated if any progress is to be made.

In a culture of *Drosophila*, which I had been inbreeding and to which I shall refer as the inbred stock, there appeared an increasing number of sterile pairs. The sterility affected primarily the females as was evident by testing them with other individuals. In the sixth generation which had descended from a single pair of grandparents of the fourth generation, 51 pairs in a total of 105 pairs were sterile. There were 47 sterile females, 3 sterile males and 1 questionable case.

It seemed probable that the defect was due to something in the hereditary mechanism rather than to some factor in the environment, since the same defect reappeared among the grandchildren which hatched at different times during a period of two months. Since the effects of inbreeding were under observation I was at first inclined to attribute this sterility to inbreeding.

STERILITY AND INBREEDING

It is a popular belief that one of the effects of inbreeding is to induce sterility. Moenkhaus and Castle, however, after many generations of inbreeding with *Drosophila* could find no evidence in favor of such a view. Nevertheless, since sterility appeared in my 'inbred' culture to such a high degree it was tempting to attribute it to inbreeding. As Moenkhaus has clearly pointed out, if in a sterile inbred stock, the sterility can be eliminated by continued inbreeding, then inbreeding can not be held to be the causative agent. This is the test to which my own case has been put, and by selecting from those families that showed the sterility in the least degree a fertile race was produced. Tables 1 to 7 show the manner in which the sterility appeared in the inbred stock and its elimination by selection.

1. Sterility of the inbred stock

TABLE 1

Sterility of the first five inbred generations

GENERATION	NO. PAIRS TESTED	PAIRS STERILE	STERILE	
			♀	♂
F ₁	16	1?		
F ₂	16	0		
F ₃	7	3		
F ₄	12	3		
F ₅ *	10	2	2	
Total.....	61	8	2	

* The ten pairs of F₅ were from a single pair of F₄.

TABLE 2

Sterility in the F₆ generation

ANCESTRY NO.	NO. PAIRS TESTED	NO. PAIRS STERILE	STERILE	
			♀	♂
25	1	1	1	0
27	11	0	0	0
28	5	3	3	0
30	32	18	17	1
31	31	16	14	2
32	10	6	5	1?
	15*	7	7	0
Total.....	105	51	47	3+1?

* Miscellaneous pairs.

TABLE 3

Sterility in the F_7 generation

ANCESTRY NO.	NO.	NO. PAIRS TESTED	NO. PAIRS STERILE	STERILE ♀	STERILE ♂
27	134	17	7	4	
30	127	5	2		
32	129	12	6		
Total		34	15	4	

TABLE 4

Sterility in the F_8 generation

ANCESTRY NO.	NO.	NO. PAIRS TESTED	NO. PAIRS STERILE	STERILE ♀	STERILE ♂
127	149	26	11	10	1
127	151	21	5	5	0
127	160	16	3	3	0
129	164	4	2	2	0
127	165	8	3	3	0
127	166	4	2	1	1
127	167	3	1	0	1
134	172	28	12	10	2
Total		110	39	34	5

TABLE 5

Sterility in the F_9 generation

ANCESTRY NO.	NO.	NO. PAIRS TESTED	NO. PAIRS STERILE	STERILE ♀	STERILE ♂
172	228	28	6	5	1
151	226	8	0	0	0
151	227	4	0	0	0
Total		40	6	5	1

TABLE 6

Sterility in the F_{10} generation

ANCESTRY NO.	NO.	NO. PAIRS TESTED	NO. PAIRS STERILE	STERILE ♀	STERILE ♂
226	232	1	0	0	0
227	229	10	0	0	0
Total		11	0	0	0

TABLE 7

Sterility in the F_{11} generation

ANCESTRY NO.	NO.	NO. PAIRS TESTED	NO. PAIRS STERILE	STERILE ♀	STERILE ♂
229	236	4	0	0	0
229	237	10	2	1	1
229	238	7	2	0	2
229	239	2	1	1	0
229	240	14	2	1	1
232	241	61	6	0	6
229	242	22	4	1	3
229	243	33	5	1	4
229	244	18	2	1	1
229	245	11	4	0	4
Total		182	28	6	22

Tables 1 to 7 show that in the beginning of the experiment only a few sterile pairs were found (table 1). Moreover, the fertile pairs were high producers (Part II, table 2) yet sterile individuals appeared in their offspring and these were largely females. At times fully half the females were affected, although there were undoubted cases in which the males were also affected.

It is to be noted in the eleventh generation, as shown in table 7, that out of 182 pairs only 6 sterile females appear. The sterility as it affected the female had been practically eliminated, for while it had been affecting 50 per cent of the females it affected at the end of the experiment less than 4 per cent. It might seem as though the character had been transferred to a certain extent to the male as 22 males out of 182 are recorded in table 7 as sterile. But the figures, as given here for the male, do not in all probability represent the actual facts in the case. Many of these males were very small, and after they had been paired for a few days practically all the males that proved sterile were crawling over the food with their wings drooping at their sides. This may or may not have prevented them from mating with the females. I was not able to follow the case further, but I have found no evidence in any of the other experiments that sterility, as it affects the females, can be shifted in heredity to the males.

That sterility is not due to inbreeding, and that selection is an effective agent in controlling it, is shown in the history of another strain, to which I shall refer as the truncate stock. This stock had been inbred for forty-two generations by Mr. Altenburg, a graduate student in the department. A great many sterile pairs were appearing in the strain and the broods were so small that it was somewhat difficult, as Altenburg told me, to keep the stock from dying out. Table 8 gives the history of the sterility as it appeared in this stock. When I took charge of the stock fully half of the pairs were sterile and yet on continued inbreeding a large percentage of the sterility disappeared. Table 11 shows that in F_{49} there were 3 sterile pairs in 21 pairs. That there were but few sterile individuals in the truncate stock at this time is also borne out by crosses that were made with fertile races. Sterility had been largely eliminated, and moreover this

had been brought about by selecting from those families that showed the least sterility (no. 17, table 9, and 17a, table 10).

It seemed to me at first that the large number of sterile individuals that appeared in this stock might bear a causal relation to the low productivity of the fertile pairs, but at the point in the experiment when the sterile individuals were eliminated there was no rise in productivity. I shall return to this question in the second part of the paper.

2. Sterility of the truncate stock

TABLE 8

Sterility of the truncate stock as it appeared in successive generations of inbreeding

	GENERATION											
	F ₁₁	F ₁₂	F ₁₃	F ₁₄	F ₁₅	F ₁₆	F ₁₇	F ₁₈	F ₁₉	F ₂₀	F ₂₁	F ₂₂
Number pairs tested.....	31	53	46	19	1	14	21	2	2	8	15	
Number pairs sterile.....	22*	27	12	6	0	2	3	0	0	1	27	
Sex.....						2♂	1♂					

* Some doubtful cases are included.

TABLE 9

Sterility of the truncate stock in F₄₅ generation

	ANCESTRY NUMBER														TOTAL
	3	5	7	8	9	15	17	21	23	25	26	33	37	52	53
No. pairs tested....	1	1	1	4	2	1	8	4	3	4	4	4	5	3	1
No. pairs sterile....	1	0	1	1	2	0	0	1	1	2	1	1	0	1	0

TABLE 10

Sterility of the truncate stock in F₄₈ generation

	NUMBER AND ANCESTRY							TOTAL
	5a	8a	17a	21a	23a	33a	52a	
Number pairs tested....	1	7	5	1	1	2	2	19
Number pairs sterile....	1	3	0	1	0	0	1	6

For the forty-seventh generation one pair was chosen from 17a. It is to be noted that no sterile individuals had appeared in the

TABLE 11
Sterility of the truncate stock as it appeared in the F₄ generation

ANCESTRY NO.	NO. PAIRS TESTED	NO. PAIRS STERILE	STERILE ♀	STERILE ♂
26	3	2	0	2
33	1	0	0	0
35	16	1	1	0
39	1	0	0	0
Total.....	21	3	1	2

last two generations of this family. Fourteen pairs were chosen from the F₄ generation, two of which were sterile. From this generation pairs were made up as shown in table 11.

It should be recalled that the truncate stock was bred at the same time and under the same conditions as the inbred stock, and while the former developed into a fertile stock the latter developed into a sterile female stock.

Attention is called here to the fact that the individuals in table 2 are the grandchildren from a single pair of the F₄ generation. Sterility appears in the different families and affects about 50 per cent of the females. Family 27 is an exception in that 11 pairs show no sterility although descended from the same grandparents. This small amount of evidence is in accord with the assumption that some individuals come through without the defect in their germ-plasm, and shows how selection has brought about its results. That one of the grandchildren in this combination had some factor which prevented the appearance in her of the defect is shown by the fact that the defect reappears among her daughters (table 3, no. 134). These numbers are too small to base a safe conclusion upon; but attention is called to them here, since the assumption is borne out in the experiments which follow.

BEHAVIOR OF STERILITY IN HEREDITY

In a study of sterility one meets at the outset a peculiar difficulty in that he cannot breed the animals that show the very defect which he wishes to study. Again there is no way by which sterile animals can be recognized except by pairing them with different mates which involves an additional amount of labor. Moreover

the same strain on inbreeding may behave differently in successive generations as has been shown in the history of the inbred and truncate stocks. An additional difficulty is met in finding a highly fertile strain against which to test a known sterile strain. In the face of these difficulties a number of detailed experiments have been required in order to determine (1) the method of transmission of the female sterility, and (2) the relation between low production and sterility in the truncate stock. I shall present first the evidence that bears directly on the method of transmission of female sterility.

TABLE 12
Sterility as it appeared in the Woods Hole stock

LOT	DATE	NO. PAIRS TESTED	NO. PAIRS STERILE	STERILE ♀	STERILE ♂
A	March 2, 1912.....	58	1	0	1
B	June 24, 1912.....	70	4	2	2
C	July 10, 1912.....	63	3	1?	2

A wild stock to which I shall refer as the Woods Hole stock was characterized by the presence of but few sterile individuals. Consequently this was good material against which to test the sterility of the inbred stock. An entirely fertile stock would be the ideal one to use but it is the experience of all who have bred these flies that a sterile pair is occasionally met without any assignable cause. Table 12 gives the history of the fertile Woods Hole stock.

At the time the following cross was made there appeared in the Woods Hole stock that was used as a control by placing under similar conditions, 4 out of 70 pairs that were sterile. There were 2 sterile males and 2 sterile females (table 12, Lot B). In order to determine the behavior of the sterility in the F_1 and F_2 generations, the inbred females were paired with the Woods Hole males. The reciprocal cross was also made. The offspring that resulted from this cross were paired together, brothers and sisters, for the F_1 generation. The grandchildren were paired for the F_2 generation. The offspring from three families of the inbred

stock were chosen to cross into the Woods Hole stock (see table 4, Nos. 149, 151, and 172). The pairs that were tested from these different families as a control show the sterility to have been present in different intensities in these families; No. 149, 11 pairs in 26 were sterile; No. 151, 5 pairs in 21 were sterile; No. 172, 12 pairs in 28 were sterile. That the controls give a fair measure of the sterility is shown by the crosses made into the fertile Woods Hole stock. These crosses are given, together with the sterility as it appeared in the F_1 and F_2 generations, in table 13 a to 13 f.

In these tables the first number gives the serial number of the cross (e.g., inbred male by Woods Hole female) and is designated as P_1 . The second column gives the number of pairs of offspring that were tested from the given family, for the F_1 generation, while the next column gives the corresponding number that were sterile. The fourth column indicates the number of pairs selected to become the parents of the F_2 generation. The succeeding columns give the corresponding number of pairs tested and sterility as it affected the different sexes.

Crosses between inbred ♂ and Woods Hole ♀

TABLE 13 a

Crosses between the inbred stock and the Woods Hole stock showing the behavior of the sterility in the F_1 and F_2 generations. Inbred ♂ No. 149 × Woods Hole ♀

P_1 NO.	PAIRS F_1 TESTED	PAIRS F_1 STERILE	ANCESTRY NO.	PAIRS F_2 TESTED	PAIRS F_2 STERILE	STERILE ♀	STERILE ♂
1	7	1	1a	12	1	0	1
			1b	10	2	2	0
2	7	0	2a	13	3	3	0
			2b	7	1	0	1
			2c	8	4	1	
3	5	0	3a	12	0	0	0
			3b	20	1	0	1
4	2	0	4a	26	9	9	0
			4b	16	10	9	1
5	5	1?	5a	10	1	1	0
			5b	20	1	1	0
			5c	12	0	0	0
Total.....	26	2		166	33	26	4

TABLE 13b

Inbred ♂ No. 172 × Woods Hole ♀

P ₁ NO.	PAIRS F ₁ TESTED	PAIRS F ₁ STERILE	ANCESTRY NO.	PAIRS F ₂ TESTED	PAIRS F ₂ STERILE	STERILE ♀	STERILE ♂
6	8	0	6a	20	0	0	0
			6b	14	4	4	0
			6c	13	0	0	0
			6d	32	0	0	0
			6e	21	2?		
7	2	1♂	7a	4	1	1	0
			7b	28	3?		
			7c	20	1?		
8	5	0	8a	9	4	4	0
9	4	0	9a	16	2	2	0
10	3	1♀	10a	18	9	9	0
11	3	0					
12	6	2♂	12a	19	2	1	1
			12b	2	0	0	0
13	9	0	13a	16	2	2	0
			13b	15	1	0	1
14	6	0	14a	2	0	0	0
			14b	3	0	0	0
15*							
16*							
17*							
Total	46	4		252	31	23	2

* Sterile

TABLE 13c

Inbred ♂ No. 151 × Woods Hole ♀

P ₁ NO.	PAIRS TESTED F ₁	PAIRS STERILE F ₁	ANCESTRY NO.	PAIRS TESTED F ₂	PAIRS STERILE F ₂	STERILE ♀	STERILE ♂
18	12	0					
19	17	0	19a	8	0	0	0
20	8	0	20a	21	0	0	0
21	8	0	21a	28	1?		
22	9	0	22a	25	2	1	1?
23	11	0	23a	15	0	0	0
24	12	1♂	24a	5	0	0	0
25	8	0	25a	10	1	1	0
26	13	0	26a	21	3?	3?	
27	9	0	27a	18	0	0	0
28	6	0	28a	20	2	1	
			28b	12	1	0	1
29	0						
Total	113	1		183	10	6	2

TABLE 13 d
Inbred ♀ No. 143 × Woods Hole ♂; reciprocal of 13 a

P ₁ NO.	PAIRS TESTED F ₁	PAIRS STERILE F ₁	ANCESTRY NO.	PAIRS TESTED F ₁	PAIRS STERILE F ₁	STERILE ♀	STERILE ♂
30	6	0	30a	50	4	2	2
31	7	0	31a	4	1?		
32	3	0	32a	20	3	3	0
33	5	0	33a	18	9	9	0
			33b	14	1	1	0
			33c	15	3	3	0
34*							
35*							
36*							
37*							
Total	21	0		121	21	18	2

* Sterile

Reciprocal: Crosses between the inbred ♀ and the Woods Hole ♂

TABLE 13 e
Inbred ♀ No. 172 × Woods Hole ♂; reciprocal of 13 b

P ₁ NO.	PAIRS TESTED F ₁	PAIRS STERILE F ₁	ANCESTRY NO.	PAIRS TESTED F ₁	PAIRS STERILE F ₁	STERILE ♀	STERILE ♂
38	10	0	38a	43	8	3	1
			38b	5	0	0	0
			38c	57	23	22	0
39	7	1♂	39a	15	6	4	
40	8	0	40a	15	1?		
			40b	17	6	6	0
41	1	0	41a	10	0	0	0
42	5	1♂+1♀	42a	24	0	0	0
			42b	35	20	17	1
43	8	0	43a	25	3	3	0
44	2	0	44a	40	5	5	0
45	2	0	45a	16	4	4	0
46*							
47*							
48*							
49*							
50*							
51*							
52*							
53*							
Total	43	3		302	76	64	2

* Sterile

TABLE 131

Inbred ♀ No. 151 × Woods Hole ♂; reciprocal of 13 c

NO. P ₁	PAIRS TESTED F ₁	PAIRS STERILE F ₁	ANCESTRY NO.	PAIRS TESTED F ₂	PAIRS STERILE F ₂	STERILE ♀	STERILE ♂
55	13	0	55a	3	0	0	0
56	9	0	56a	51	3	2	
57	13	0	57a	16	1	1	0
58	9	0	58a	22	1	1	0
59	11	0	59a	14	0	0	0
60	11	1♂	60a	24	8	8	0
61	9	0	61a	6	3	2	1
62	4	1?	62a	22	1	1	0
			62b	4	0	0	0
63	8	0	63a	4	2?	1?	1?
			63b	4	0	0	0
64	11	0	64a	50	0	0	0
65	4	0	65a	32	3	1	2
			65b	6	0	0	0
			65c	5	2		
66							
67							
68							
69							
70							
71							
72							
73							
74*							
75*							
76*							
77*							
Total.....	102	2		262	24	17	3

* Sterile

This experiment brings out the following facts. In the sterile female stock (inbred) used as a control 39 pairs out of 110 pairs were sterile. In the Woods Hole stock 4 pairs out of 70 pairs were sterile. In the F₁ generation only 12 pairs in 351 proved sterile. Of those tested 5 were females, 4 were males. In the F₂ generation 1286 pairs were tested, 195 of which were sterile. There were 153 sterile females and 15 sterile males. The sterility of the female reappeared in both the cross and the reciprocal, indicating that both the brothers and sisters of the affected

females were capable of transmitting the defect. The control shows that family No. 151 of the inbred stock was the least affected, and it is to be noted that sterility reappears among the grandchildren of this family in less intensity than among the grandchildren of the other two families. This fact seems to indicate that the intensity to which the sterility reappears in the F_2 generation bears a causal relation to the intensity in which it entered the cross.

Crosses between inbred, truncate and Woods Hole stock

In the sixth generation of the inbred stock, when the sterility of the females appeared in its greatest intensity (50 per cent; table 2), crosses were made with the Woods Hole stock and also with the truncate stock. The truncate stock was also crossed with the Woods Hole stock, and since the truncate stock at this time was a relatively fertile stock, it serves as an excellent control for the female sterility of the inbred stock. This experiment then consisted of three crosses, together with their reciprocals and controls: (1) The inbred stock by the Woods Hole stock; (2) The inbred stock by the truncate stock; (3) The truncate stock by the Woods Hole stock.

The control on the Woods Hole stock used in this experiment shows that one sterile male occurred in 58 pairs (table 12, Lot A).

TABLE 14a

Crosses between the inbred stock and the Woods Hole stock, showing the behavior of sterility in the F_1 and F_2 generations. Woods Hole ♀ × inbred ♂ No. 31

NO. P_1	PAIRS TESTED F_1	PAIRS STERILE F_1	ANCESTRY NO.	PAIRS TESTED F_2	PAIRS STERILE F_2	STERILE ♀	STERILE ♂
36*							
37	22	1	37a	17	0	0	0
			37b	13	0	0	0
			37c	16	0	0	0
38	4	0	38a	10	0	0	0
39	1	0	39a	16	2	1	1
40	0						
Total	27	1		72	2	1	1

* Sterile ♂

The truncate stock shows only a slight degree of sterility (table 8, F_{19}). The crosses themselves bear out this statement, for, out of the 24 pairs, all are fertile (table 16). Two died, however, before their fertility could be established and are not recorded in the table. Of the 251 pairs tested in the F_1 generation there are only 2 sterile individuals; a male and a female. The behavior of the sterility is the same in the cross as in the reciprocal. If the sterility behaves like a character which segregates and recombines

TABLE 14b
Woods Hole ♂ × inbred ♀ No. 31; reciprocal 14 a

NO. P_1	PAIRS TESTED F_1	PAIRS STERILE F_1	ANCESTRY NO.	PAIRS TESTED F_2	PAIRS STERILE F_2	STERILE ♀	STERILE ♂
41	0	0	0	0	0	0	0
42	7	0	42a	12	7	4	
43	24	0	43a	24	4	2	1
			43b	20	0	0	0
			43c	40	3		1
			43d	33	6	3	
44*							
45*							
Total.....	31	0		129	20	9	2

* Sterile ♀

TABLE 15 a
Crosses between the inbred stock and the truncate stock showing the behavior of sterility in the F_1 and F_2 generations. Inbred ♀ No. 31 × truncate ♂ No. 35

NO. P_1	PAIRS TESTED F_1	PAIRS STERILE F_1	ANCESTRY NO.	PAIRS TESTED F_2	PAIRS STERILE F_2	STERILE ♀	STERILE ♂
25	16	0	25a	16	6	3	1
			25b	36	0	0	0
			25c	28	9	4	4
			25d	18	6	4	2
			25e	33	3		
26*							
27*							
28*							
29*							
Total.....	16	0		131	24	11	7

* Sterile ♀

TABLE 15 b

Inbred ♂ No. 31 × truncate ♀ No. 35; reciprocal 15 a

NO. P ₁	PAIRS TESTED F ₁	PAIRS STERILE F ₁	ANCESTRY NO.	PAIRS TESTED F ₂	PAIRS STERILE F ₂	STERILE ♀	STERILE ♂
31							
32							
33	8	0	33a	46	4	2	
			33b	29	15	7	1?
			33c	48	9	7	
34	19	0	34a	24	1	1	0
			34b	39	3	2	1
			34c	12	5	2	3
35	11	0	35a	48	6	3	1
			35b	31	3	1	
			35c	38	22	9	1?
Total.....	38	0		315	68	34	7 .

TABLE 16 a

Crosses between Woods Hole stock and truncate stock showing behavior of sterility in F₁ and F₂ generations. Woods Hole ♀ × truncate ♂

NO. P ₁	ANCESTRY NO. P ₁	PAIRS TESTED F ₁	PAIRS STERILE F ₁	ANCESTRY NO.	PAIRS TESTED F ₂	PAIRS STERILE F ₂	STERILE ♀	STERILE ♂
18	35	10	0	18a	7	1	1	0
				18b	14	0	0	0
19	35	15	0	19a	22	2	2	0
				19b	12	3		
20	26	9	0	20a	15	8	4	4
				20b	17	2	2	0
21	26	18	0	21a	19	0	0	0
				21b	25	1?		
				21c	8	0	0	0
22	35	11	0	22a	24	2	1?	1
				22b	32	4	4	0
23	35	9	0	23a	7	0	0	0
				23b	5	0	0	0
24	35	22	1?	24a	23	0	0	0
				24b	13	2	1	1
		54*	0					
Total.....		148	1		243	25	15	6

* Pairs chosen at random from the above crosses.

in the germ plasm we should expect it to reappear only slightly intensified in this case among the grandchildren. A study of the 528 pairs tested in the F_2 generation verifies the expectation. This case shows that sterility as it reappears among the grandchildren bears a more or less definite relation to the degree to which it was put into the cross. The behavior of the sterility of the truncates at this time is significant for their earlier history shows that half the pairs were sterile. As stated, it was at first my opinion that sterility was inherent in the strain, and that it bore a causal relation to low productivity. But the above results seem to show that sterility and low production of fertile brothers and sisters are two entirely different things. Such a

TABLE 16b
Woods Hole ♂ × truncate ♀; reciprocal 16 a

NO. P ₁	ANCESTRY NO. ♀ P ₁	PAIRS TESTED F ₁	PAIRS STERILE F ₁	ANCESTRY NO.	PAIRS TESTED F ₂	PAIRS STERILE F ₂	STERILE ♀	STERILE ♂
1	35	13	0	1a	12	3?	2?	1?
				1b	25	1	0	1
2	35		0		0	0	0	0
3	35	10	0	3a	14	0	0	0
				3b	7	1	1	0
4	35	12	0	4a	16	1?	1?	0
				4b	20	1	1	0
5	35	25	0	5a	20	2	2	0
				5b	19	0	0	0
6	35							
7	35							
8	33	4	1♂	8a	14	1	0	1?
				8b	38	1	1	0
9	33							
10	26	11	0	10a	19	2?		1
				10b	6	2?		1
				10c	17	0	0	0
11	26	7	0	11a	8	1	0	1
				11b	11	0	0	0
12	33	8	0	12a	13	0	0	0
				12b	26	11	0	11
13	34							
14	34	5	0					
17	35	8	0					
Total.....		103	1		285	27	8	17

view paved the way for a study of the low production of the truncates, which is dealt with in the second part of this paper.

It is to be noted in 12 b (table 16 b) of this cross that 11 pairs out of 26 are sterile. This proved to be due to the males. Twenty-eight other males from this family were tested with the hope of establishing a sterile male strain. Seventeen of the 28 were sterile. This gave promise of yielding a sterile male line; but on inbreeding a few pairs the sterility vanished as suddenly as it had appeared. This is the only case in all the experiments in which sterility affected the males to any appreciable degree.

Let us turn now to the evidence which this experiment gives on the transmission of female sterility. A study of the tables where the sterile female strain (inbred stock) was tested against the truncate and Woods Hole stocks shows that the sterility reappeared again after skipping a generation and that it affected primarily the females. The defect reappears in both the cross and the reciprocal. A great deal of weight is to be attached to this evidence since the sterility did not appear to any appreciable degree when the truncate stock was crossed to the Woods Hole stock. Moreover, the sterility as it was affecting the female was now at its maximum. In table 14 a there are three inbred males that did not transmit the defect. An apparent explanation is found on the assumption, which is borne out by all the evidence presented, that the defect behaves like a unit character and accordingly some families are not affected.

Crosses between inbred and truncate stock

Before proceeding to a summary of these sterile cases I wish to give the results of an experiment in which the inbred stock was crossed into the truncate stock. Sterility was present in both stocks at this time. Tables 17 a and 17 b give the result of the sterility as it appeared in the F_1 and F_2 generations.

Table 1, F_3 , gives the control for the inbred stock while table 8, F_{46} , gives the control for the truncate stock. The inbred strain up to the time this cross was made had been relied upon as a fertile strain. That sterility had appeared in this stock and

TABLE 17a

Crosses between the inbred stock and the truncate stock showing the behavior of sterility in the F_1 and F_2 generations. Inbred ♀ × truncate ♂

NO. P_1	NO. PAIRS TESTED F_1	NO. PAIRS STERILE F_1	NO. PAIRS TESTED F_2	NO. PAIRS STERILE F_2	STERILE ♀	STERILE ♂
18	3	0	7	1		
19	3	0	6	2		
20	6	0	9	3		
21	2	0	0	0		
22*						
23*						
24	4	0	9	3	1	2
25*						
26*						
27*						
28*						
29	8	1	33	0		
30	6	0	14	3		
31	0	0	0	0		
32	8	0	3	1		
33	1	0	0	0		
34	7	0	3	1		
Total.....	48	1	84	14	1	2

* Sterile

TABLE 17b

Inbred ♂ × truncate ♀; reciprocal 17a

NO. P_1	NO. PAIRS TESTED F_1	NO. PAIRS STERILE F_1	NO. PAIRS TESTED F_2	NO. PAIRS STERILE F_2	STERILE ♀	STERILE ♂
1	7	1	4	3		
2*						
3	6	0	8	5		
4	0	0	0	0		
5	6	0	22	4		1
6	0	0	0	0		
7	10	0	4	4	4	
8*						
9	11	0	11	5		
10	13	2	28	3	1	
11*						
12	7	0	17	7	0	2
13	4	0	2	0		
14	6	0	3	1		
Total.....	70	3	99	32	5	3

* Sterile

that it was also present in the truncate stock at this time is shown not only from the controls but also from the crosses themselves. It looks at first sight as if the sterility reappeared in a higher degree in the reciprocal than it did in the cross, since it occurs in 32 cases out of 99 in the reciprocal while in the cross the sterility occurs in 14 pairs of the 84 pairs tested. It is to be noted, however, that the pairs as made up are not equally distributed, for, number 29 of the cross has 33 representatives none of which are sterile. If correction is made for this we have 14 sterile pairs in 51 or 28 per cent which corresponds fairly well with the 33 per cent of sterility as it occurred in the reciprocal cross. The fact that 33 pairs of number 29 showed no sterility is significant as has already been pointed out; for, it shows that some families do not show the defect, while in closely related families the defect may be present to a high degree.

In table 18 I have compiled the results of all the experiments that deal with the transmission of sterility. A census of the whole situation shows that of 417 pairs set aside as controls 113 pairs were sterile. Twenty-seven per cent of the controls were sterile. There were 84 sterile females and 14 sterile males. Of the 149 crosses made between the sterile and fertile strains 35 pairs, or 24 per cent, were sterile. If the sterility behaves anything like a recessive character the expectation is that sterility will not appear in the F_1 generation. A total of 832 pairs tested in the F_1 generation verifies the expectation as only 19, or 2.3 per cent, are sterile. These seem to be chance occurrences as both sexes are equally affected—5 females and 6 males. If one could find an absolutely fertile race against which to test the sterility, it is altogether probable that no sterility at all would appear in the F_1 generation. The sterility as it appeared here in the F_1 generation apparently gives a fair measure of the degree to which the sterility entered into the experiment from the strain against which the sterility of the sterile strain was tested.

In the F_2 generation we find that the sterility as it affects the female reappears. Of the 2644 pairs tested, 407 pairs, or 16 per cent, proved sterile. There were 237 sterile females and 60 sterile males. The rather large number of males given here in-

TABLE 18—Continued

	70	4	2	2	5	0	0	0	26	1+1?	—	—	166	33	26	4
III a	70	4	2	2	5	0	0	0	26	1+1?	—	—	166	33	26	4
	110	39	34	5	8	4	—	1	21	0	0	0	121	20+1?	18	2
	No. 149	26	11	10	1	8	—	—	46	4	2	2	252	25+6?	23	2
III b	70	4	2	2	12	3	—	—	—	—	—	—	—	—	—	—
	110	39	34	5	16	8	—	—	43	3	2	1	302	76	64	2
	No. 172	28	12	10	2	0	0	0	113	1	0	1	183	6+4?	2+3?	1+1?
III c	70	4	2	2	12	0	—	—	—	—	—	—	—	—	—	—
	110	39	34	5	23	4	—	—	102	2	—	1	262	22+2?	16+1?	2+1?
	No. 151	21	5	5	0	—	—	—	—	—	—	—	264	407	237	60
Total.....	417	113	84	14	140	35	6	2	832	19	5	6	2644	407	237	60
Per cent.....		27			24					23					16	
Total in cross.....					68	11	4	1	401	12	2	4	1188	159	79	36
Total in reciprocal.....					81	24	2	1	431	7	3	2	1456	248	158	24

Total number of sterile cases in these three experiments, 574.

Total number of pairs tested in these three experiments, 4042.

cludes 15 sterile ones that occurred in one family and was probably due to a chance combination.

A glance at the summary given in table 18 will show that the sterility as it affected the female is practically the same whether inherited through the male or female. I think that the 574 cases of sterility recorded in this summary of 4042 pairs tested gives a faithful picture of the actual facts in the case as seen on a minor scale in each of the experiments and in the history of the inbred stock. The evidence is conclusive that sterility as it affects the female may be present in different intensities in the different families and that female sterility is transmitted by the fertile brothers and sisters of the affected females to the granddaughters but not to the grandsons. This character behaves like a recessive Mendelian character in that it disappears in F_1 and reappears in F_2 .

CROSSES INVOLVING A SEX-LINKED FACTOR

In this experiment, which unfortunately was not carried out on a very extensive scale, the white-eyed stock was used. This stock arose from a mutant of the inbred stock. Females were

TABLE 19

Behavior of sterility in F_2 generation of No. 12 b; control of the supposed sterile male strain

	NUMBER							TOTAL
	1	2	3	4	5	9	11	
Number pairs mated....	2	4	8	12	3	11	3	43
Number pairs sterile....	0	0	1	0	0	2	0	3

paired with the fertile males from No. 12 b, table 15. The reciprocal cross was not made. Family No. 12 b arose as a cross between the Woods Hole and truncate stocks. One-half of the males from this particular combination were sterile. It was my hope that this would develop into a male-producing strain; but the sterility vanished suddenly.

A number of flies from No. 12b were allowed to breed in order to obtain the next generation. From this eighteen pairs were

drawn off and tested in F_1 generation. Much to my surprise, all proved fertile. From 5 of these, seven pairs were made up in the F_2 , as shown in table 19.

As a control of the white-eyed stock eight pairs were tested all of which were fertile.

Fifteen males, brothers of the sterile males whose fertility had been established, were crossed with 15 white-eyed females; all the pairs proved fruitful. Offspring from five of these crosses were paired as shown in table 20. All the males represented in the 62 pairs in the table have white eyes like the mother, while all the females have red eyes like the father, since this is a case of sex-linked inheritance. On inbreeding, four classes of offspring will be produced in the next generation: (1) white-eyed males; (2) red-eyed males; (3) white-eyed females; (4) red-eyed females. The four classes were about equally represented. The virgin females were placed with a number of males; the males with a number of virgin females. The result is given in table 21.

TABLE 20

Sterility as it appeared among the children of the crosses; F_1 generation

	NUMBER					TOTAL
	1	2	3	4	5	
Number pairs tested ..	16	21	5	6	13	62
Number pairs sterile ..	3	9	0	1	0	13

TABLE 21

Sterility as it appeared in the F_2 generation, Offspring from two of the 21 pairs of No. 2, table 20

Family No. 2 a					Family No. 2 b				
	CLASS					CLASS			
	Red ♂	Red ♀	White ♂	White ♀		Red ♂	Red ♀	White ♂	White ♀
Number tested....	18	19	10	10	Number tested....	14	21	13	20
Number sterile ..	3	0	1	0	Number sterile....	2	10+1?	1?	13+2?

The figures given in this experiment are very small and the source of the sterility doubtful since the controls throw no light upon it. It is to be noted, however, that both classes of females are affected in family No. 2 b. In the case of the white-eyed females more than 50 per cent are affected.

To analyze the evidence from the foregoing experiments let it be assumed that a gene responsible for the functioning of the oviduct is carried by the X-chromosome and that in the sterile individuals this gene has changed so that the oviduct fails to function. In analogous cases when the female is affected both X's are affected but she remains normal so long as only one X is affected. When the affected X goes into the males they should not be sterile because the male has no oviduct. But such a male will be able to transmit the defect. To follow the argument, let us make three assumptions.

a. If the defect in this experiment came from the white-eyed grandmother then her germinal make-up would be expressed by the formula $w w X x$, in which the x represents the affected chromosome. Since the red-eyed male did not contain the defect his formula is $R X O$. Crossing these two individuals gives:

$$\begin{array}{r} w x - w X \\ R X - O \quad \text{Gametes} \\ \hline w R X x - w R X X - w x O - w X O \quad F_1 \text{ Red-eyed females and white-eyed males, none of which show sterility.} \end{array}$$

If by chance the flies chosen have affected genes, we get:

$$\begin{array}{r} w x - R X \\ w x - O \quad \text{Gametes} \\ \hline w w x x - w R X x - w x O - R X O \quad \text{Flies of the } F_2 \text{ generation; only white-eyed females are sterile} \end{array}$$

But this does not agree with the facts of the experiment.

b. If the defect was brought in by the red-eyed grandfather and the female is free from it, then it follows:

$$\begin{array}{r} w X - w X \\ R x - O \quad \text{Gametes} \\ \hline w R X x - w X O \quad F_1 \text{ Red-eyed females, white-eyed males; all fertile.} \end{array}$$

On mating the F_1 the following results:

$wX - Rx$	Gametes
$wX - O$	
<hr/>	
$wwXX - wRXx - wXO - RxO$	F_2 White-eyed females, red-eyed females, white-eyed males, red-eyed males; no sterile individuals.

This does not accord with the sterility as shown in this experiment.

c. If the defect is brought in the X-chromosome of both grandparents, it follows:

$w x - w X$	Gametes
$R x - O$	
<hr/>	
$w R x x - w R X x - w x O - w X O$	F_1 Red-eyed females, white-eyed males. Half of the females sterile. •

On mating the F_1 the following results:

$w X - R x$	Gametes
$w x - O$	
<hr/>	
$w w X x - w R x x - w X O - R x O$	F_2 White-eyed females and red-eyed females; white-eyed males and red-eyed males; red-eyed females sterile.

This accords with the appearance of the eye colors but not with the appearance of the sterility, since there was practically none in F_1 .

These three assumptions exhaust the possibilities on the hypothesis that the defect is carried by the X-chromosome and yet none of them meets the facts in the case of this experiment. I conclude, therefore, that sterility as it affected the female in this experiment was not transmitted by the X-chromosome. There is a possibility, however, that the factor involved is in the sex chromosome but so far from W that it crosses over freely with it.

DISCUSSION

The foregoing series of experiments brings out the following facts. (1) Sterility as it appeared in the inbred stock affected primarily the females. The males may also be sterile but it seems probable that the sterility of the male bears no causal relation to the sterility that affects the female. (2) The defect is germinal

in character in that it is strongly transmissible through inheritance, behaving like a character that segregates. The evidence goes to show that the character in all probability is not carried by the X-chromosome. (3) The sterility as it affects the female can be eliminated by selection. Inbreeding, as such, does not appear to be the causative agent in producing this kind of sterility.

A close study of the females revealed the fact that they were not laying eggs. The abdomen swelled until it was very large and in many cases the ovipositor protruded some distance beyond the body. The female could not deposit her eggs. A score or more females were sectioned and these sections showed eggs present in abundance. In fact, a mere examination of the fly after it had been mashed on a glass slide showed in all cases, apparently well-formed eggs in large numbers. I have examined scores in this manner after they had been tested with a number of males and except only one doubtful case I have found that all had eggs. The exact nature of the defect cannot be stated until more attention is given to a study of sections, but the defect is of such a kind that it prevents her from laying her eggs. The possibility that parasites were the source of the trouble suggested itself. Sections show, however, that these flies are remarkably free from parasites in so far as one can make out with ordinary stains, and the behavior of sterility in transmission, moreover, shows the futility of such an hypothesis.

In the case of the fertile female one has merely to bring pressure to bear upon the abdomen to cause her to expel an egg. The sterile females will not respond to such treatment. On the contrary, the wall of the abdomen will burst, and allow the eggs to roll out, while none will pass through the oviduct. Whether or not the eggs are ever fertilized I cannot say. I have frequently seen the sterile female copulate and I have planted scores of eggs (obtained by opening the female) on banana but never has a single larva emerged.¹ All attempts at artificial fertilization proved a failure.

¹ A fertile female placed on a very poor grade of banana may refuse to lay her eggs for some time. She holds them as long as she can but after a few days will lay them.

It is to be recalled that Castle in his cultures found both males and females sterile. Moenkhaus found in his strain, in 64 cases tested, that the males alone were sterile.² Moenkhaus in attempting to harmonize Castle's results with his own points out that the two cases can not with certainty be compared since Castle took no account of the emergence of larvae but merely the production of pupae. In other words, Castle's measure of productiveness was eggs that gave rise to pupae; Moenkhaus's measure, the emergence of larvae. From my studies this difference seems rather apparent than real since in only one or two very questionable cases have I found that larvae after emerging from the egg were unable to develop to the adult stage. I do not wish to be misunderstood on this point. Not every zygotic combination, as is evident from my later studies, results in the production of a well formed fly. In fact, the percentage, as I have been able to show, may be very low. Whether the sperm enters the egg, or having entered the combination dies, is another question. The fact holds in my strains that if the larvae emerge some of them develop to the hatching stage.

I wish to point out that my results and those of Castle are more likely to be harmonized on the assumption that a distinction exists between fertility and sterility. Castle and Moenkhaus seemed to consider that the defect related to sperm and egg structure. I infer from Castle's paper that he supposed that sterile females occurred more frequently in a strain inclined to low productivity; that low fertility and complete sterility as it affects the female are causally related. I interpret him to mean that there is a range in the capacity of the eggs for fertilization by good sperm, extending from zero (or complete sterility) to high fertility. In my strain no such condition is involved; but it is conceivable that the egg-laying power ranges in degree from zero to complete productivity. If so some of the sisters of the sterile females

² He mentions one exception which from the present standpoint seems significant. "In one instance I found among a brood, beside a sterile male, two females that failed to deposit eggs although eggs were evidently present in the oviducts." It seems likely that this is the same defect that I have found so prevalent in my cultures.

should be 'low producers. This would seem to correspond to Castle's results but in my case we meet with a difficulty at the outset on such an assumption for it appears that the egg-laying power of the affected female is totally abrogated. My evidence will not deny the possibility of transitional stages but it is not clear how an egg-laying mechanism should be able to expel a few eggs and not all.

Critical evidence is hard to obtain on this point because low production may be due to a variety of causes. Attention is called to the truncates, in which there was no rise in productivity as the sterile individuals were practically eliminated.

The history of the inbred stock allows us to form a more reliable opinion, for the total output of offspring at first was much greater. In table 2, Part 2, we get results that look as though the productivity is running down as the sterile females appear. The table, however, does not convey all the facts of the experiment, for in the F_6 generation where 65 offspring is recorded as the average per pair, some of the flies failed to emerge from the pupae. The generations which precede and follow show more nearly the actual facts in the case, namely, a gradual decrease in productivity. That this was a high producing strain in the beginning of the experiment there can be no doubt; for in the second generation the average for sixteen pairs is 365 offspring. The low production in the first generation is probably due to inexperience in handling, as half of the females were dead at the end of two weeks. The production gradually fell despite the fact that facility in handling became more and more perfected. In the F_{14} generation the production had dropped to 159 per pair despite the fact that the sterile females had been practically eliminated. This led to an investigation of the defect. It was found by isolating the eggs of the females of the F_{14} generation, which had been placed with a number of their own males, that only 32 per cent hatched. And yet in the same experiment under identical conditions 58 per cent of the eggs of the F_{14} female hatched when paired with the males from the truncate stock. Moreover, the males of the F_{14} generation were able to fertilize 52 per cent of the eggs of the females of the truncate stock.

These facts make it evident that incompatibility of some kind had arisen between the egg and sperm of the inbred stock. It is not evident that this is in any way related to the appearance of the affected females in this strain.

In regard to the method of transmission of the sterility as it affects the female, Mast, one of Castle's students, came to the conclusion that "An entirely fertile male may transmit partial or complete sterility of the female sex as a racial character to his granddaughters though not apparently to his daughters." Since Castle and Mast's paper deals primarily with the effects of inbreeding, the data upon which these conclusions are founded are very small. As will be seen from my tables, this method of transmission has been verified many times, the exception to be taken here is that in all likelihood the partial fertility bears no relation to the complete sterility. The reciprocal cross that led Mast to the above conclusion

. resulted in one case in the production of females half of which were sterile and the other half were of low productiveness. This case shows that a female of a race inclined to sterility may transmit that character directly to her cross-bred offspring. This difference in heredity through the two sexes would seem to indicate that sterility of the female is dependent upon egg structure, the eggs produced by mothers of a fertile race always yielding fertile daughters. But the eggs of cross-bred females, whose father was of an infertile race, produce some of them fertile, some infertile females.

Such a method of transmission is not borne out in my cases. The females transmit sterility to the granddaughters in the same way as the males and it is certain that the defect skips a generation. Castle and Mast's paper gives us no certain proof as to the defect in the female. It seems to be assumed that she is laying eggs but that these eggs are incapable of fertilization. If this is true her sterility cannot be compared at all to that of the females in my case. In all my experiments, however, I have never found but one or two questionable cases in which the female having laid her eggs proved sterile. In fact, toward the end of the experiments it was easy to tell which sex was at fault by observing whether or not the female was laying her eggs. While this was never made the final test it always held good that when the female

laid eggs which did not develop the male was found to be at fault. Some of the eggs of such a female always hatched on placing her with fertile males. It may or may not be that Castle's case and mine are similar since they behave somewhat differently in heredity. Since Castle's paper deals primarily with another problem, the data upon which the above conclusions in regard to the method of transmission are based are too small to base any definite conclusions upon, since the influence that may have entered into the experiment from the other sex is not with certainty under control. A glance at Mast's last conclusion will show that the male also may have been at fault.

CONCLUSIONS

1. One kind of sterility in *Drosophila ampelophila* is due to some defect, probably in the oviduct of the female, so that she is unable to deposit her eggs.
2. The defect is transmissible through inheritance by at least some of the brothers and sisters of the affected females when mated to a fertile race, to the granddaughters, but apparently not to the sons or daughters or grandsons. It is therefore recessive and affects females only.
3. The process of inbreeding brothers and sisters cannot be held to be responsible for this condition, but probably serves to bring it out when latent in a strain by making the necessary combinations.
4. The character seems amenable to selection and can be made to affect fully 50 per cent of the females or can be practically eliminated by making the proper selections.
5. It seems very probable that sterility as it affects the male bears no causal relation with sterility as it appears in the female.
6. The defect in the female behaves after the manner of a Mendelian character in that it reappears after skipping a genera-

tion. The normal function is dominant to the negative condition. An unaffected male can transmit something as a dominant character which causes the normal egg deposition of his daughters.

7. The defect is sex-limited in the sense that it affects the female only but it is probably not sex-linked in the sense that it is carried by the X-chromosome. If so, it is such a distance from W that it crosses over more or less freely with it.

I wish to express to Professor Morgan my appreciation of his interest throughout the present study. I am also grateful for the generous gift of the Dyckman Fund which the Department voted me for 1912-1913.

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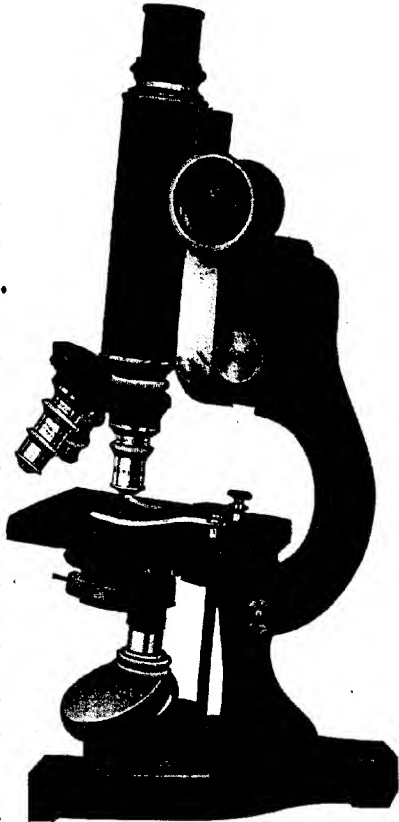
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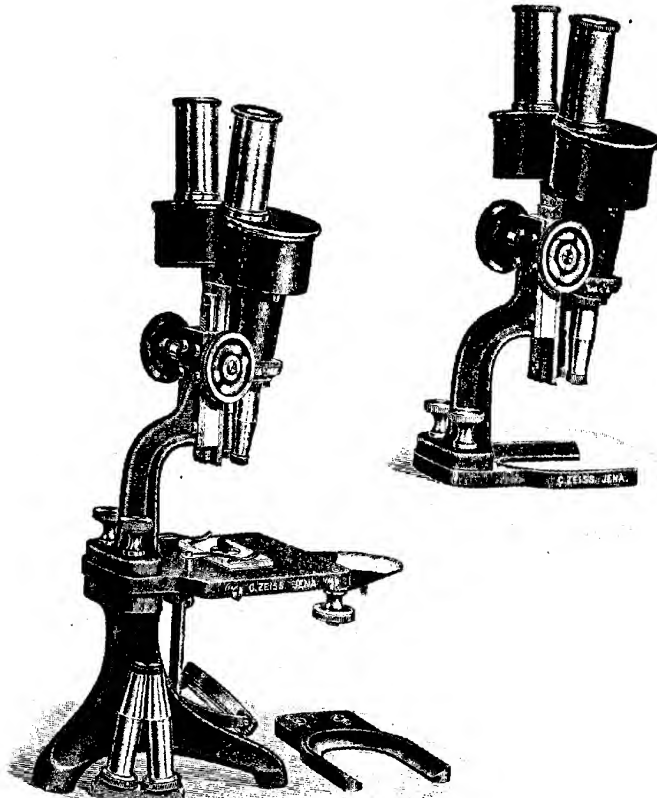
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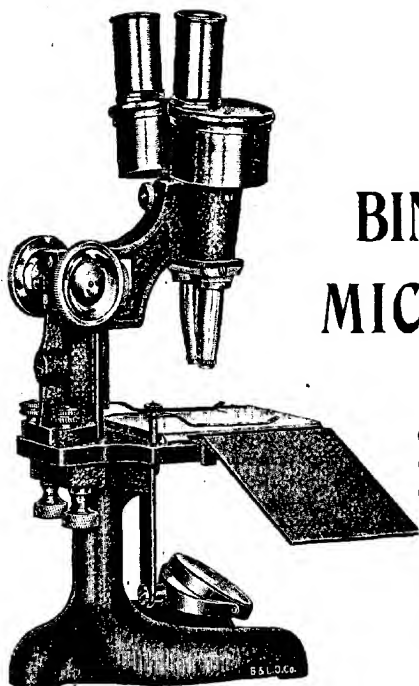
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FERTILITY AND STERILITY IN DROSOPHILA AMPELOPHILA

II. FERTILITY IN DROSOPHILA AND ITS BEHAVIOR IN HEREDITY

ROSCOE R. HYDE

From the Zoological Laboratory, Columbia University

NINE FIGURES

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INTRODUCTION

The first study demonstrated that the sterility there dealt with is due to some defect probably in the oviduct of the female that prevents her from laying eggs. Since this defect is transmitted by males and heterozygous females, the defect when once in a strain constantly recurs. It is evident that this kind of sterility in the female is an entirely different thing from infertility in the sense that eggs laid are not fertilized or if fertilized do not develop.

It is evident from Part I that the low fertility of the truncate stock would not be explained by the presence of the sterile individuals for after sterility had been practically eliminated the

truncates produced no more offspring per fertile pair than at the beginning of the experiment when half of the pairs were sterile. Neither can the low productivity of the truncates be explained entirely because of the shortened length of life, although this is one factor in the result.

The truncates differ in many ways from the wild stock:

1. The flies give rise to a small number of offspring per pair. Table 1 gives the number of offspring produced through several generations of inbreeding. Despite all the care in breeding that could be exercised no way was found to increase the output.

2. The truncate flies do not live as long as the wild fly. The length of life is in general about half that of the inbred stock which was used in the control. The two strains were bred under identical conditions (compare tables 1 and 2).

3. Truncates, as the name implies, have the ends of the wing squared instead of rounded. The wings extend only to the tip of the abdomen. The flies will not breed true, for they produce

TABLE 1
History of truncate stock through seventeen generations of inbreeding

	GENERATION												
	F ₁₂	F ₁₁	F ₁₀	F ₉	F ₈	F ₇	F ₆	F ₅	F ₄	F ₃	F ₂	F ₁	F ₀
No pairs tested.....	31	53	46	19	1	14	21	2	2		8		15
No pairs sterile.....	22?	27	12	6	0	2	3	0	0		1		2?
No pairs from which off- spring were counted...	9	26	34	13	9	9	8				7		13
Truncate ♂.....	173	855	684	266	—	79	182				182		218
Truncate ♀.....	212	911	696	292	—	99	202				162		228
Long ♂.....	41	123	136	29	—	10	37				31		66
Long ♀.....	43	117	102	32	—	14	25				24		57
Total ♂.....	214	978	820	295	14	89	219				213		284
Total ♀.....	255	1028	798	324	14	113	227				186		285
Grand total.....	469	2006	1618	619	28	202	446				399		569
Average.....	52	77	47	48	28	22	56				57		51
Average life of ♂ in days.....	—	—	26	27	—	26	26				27		23
Average life of ♀ in days.....	—	—	16	25	—	17	25				19		17

some offspring with wings like those of the wild fly; at least this has held true through many generations of inbreeding truncate brothers and sisters. The long winged flies from truncates throw in turn some truncates.

HISTORY OF THE LOW-PRODUCING TRUNCATE AND THE HIGH-PRODUCING INBRED STOCKS

In order to bring into sharp contrast the difference that exists in the productivity of these two strains I have compiled in tables 1 and 2 the data of these two strains through several generations of inbreeding. The truncates gave on an average about 50 offspring per pair, and the inbred stock about 200 offspring per pair. It has been stated that the inbred flies live longer than the truncates, but this difference, as the tables show, will not account for the marked difference in production.

Table 2 gives the history of the wild inbred stock described in Part I. This was bred parallel to the truncates in table 1 and was subject to exactly the same environmental conditions.

TABLE 2
History of the inbred stock

	GENERATION													
	F ₁	F ₂	F ₃	F ₄	F ₅	F ₆	F ₇	F ₈	F ₉	F ₁₀	F ₁₁	F ₁₂	F ₁₃	F ₁₄
No. pairs tested	16	16	7	12	10	105	34	100	40	11	82	12		16
No. pairs sterile	1?	0	3	3	2	51	15	39	6	0	28	1		1
No. pairs from which offspring were counted	13	13	4	9	8	11	11							14
Males produced	975	2374	430	851	708	336	629							1098
Females produced	1034	2406	395	859	766	310	677							1142
Total	2009	4780	834	1713	1474	646	1306							2240
Average	155*	368	209	190	184	65†	119							159
Days ♂ lived	—	45	42	41	36	45	34							45
Days ♀ lived	—	37	35	33	37	35	30							26

* The low production in this case was due to inexperience in handling. Half of the females were dead at the end of the second week.

† Some of the flies failed to emerge from the pupae.

CROSSES BETWEEN THE LOW-PRODUCING TRUNCATE STOCK
AND THE HIGH-PRODUCING WILD STOCKS

The following experiments were carried out in order to determine whether the egg or sperm was responsible for the low fertility of the truncates.

Females from the low-producing truncate stock were paired to males from the high-producing inbred stock. The reciprocal cross was also made. Since the number of offspring bears a more or less direct relation to the length of life of the pairs, data on the length of life is also given. Tables 3, 4 and 5 give the result of the first experiment.

This experiment shows that the low-producing truncates were producing an abundance of fertile sperm, since each was capable of fertilizing on the average 428 eggs of the female of the high-

TABLE 3a

Showing crosses between the low-producing truncates and the high-producing inbred stock

Truncate ♀ × inbred ♂

NO.	CRES- CENT ♂*	CRES- CENT ♀	LONG ♂	LONG ♀	TOTAL ♂	TOTAL ♀	GRAND TOTAL	LIFE OF ♂ IN DAYS	LIFE OF ♀ IN DAYS
1	5	7	101	119	106	126	232	56	26
2†	—	—	—	—	—	—	—	15	41
3	0	0	53	71	53	71	124	15	31
4	—	—	—	—	—	—	—	?	15
5	10	11	146	162	136	173	329	41	13
6†	—	—	—	—	—	—	—	43	8
	2	5	36	54	38	59	97	41	7
8†	—	—	—	—	—	—	—	63	7
10	10	6	185	187	195	193	388	56	26
11†	—	—	—	—	—	—	—	34	13
12	0	0	134	113	134	113	247	35	35
14	6	4	80	61	86	65	151	16	16
16	6	2	78	103	84	105	189	37	24
17	2	0	11	14	13	14	27	44	5
Total.....	41	35	824	884	865	919	1784	496	267
Average.....	—	—	—	—	—	—	198	38	19

* 'Crescent' as recorded in table 3 are longwinged flies with a small crescent-shaped piece cut out of the inner margin at the end of the wing. They are a new class that arise on crossing the truncates with wild stock.

† Sterile.

TABLE 3b
Reciprocal: Truncate ♂ × inbred ♀

NO.	CRES- CENT ♂	CRES- CENT ♀	LONG ♂	LONG ♀	TOTAL ♂	TOTAL ♀	GRAND TOTAL	LIFE OF ♂ IN DAYS	LIFE OF ♀ IN DAYS
18	5	27	332	316	337	343	680	49	59
19	5	4	130	136	135	140	275	?	53
20	0	2	283	335	283	337	620	66	66
21	0	0	191	173	191	173	364	33	?
22*	—	—	—	—	—	—	—	—	—
23*	—	—	—	—	—	—	—	63	63
24	5	17	185	189	190	206	396	26	24
25*	—	—	—	—	—	—	—	41	11
26*	—	—	—	—	—	—	—	33	41
27*	—	—	—	—	—	—	—	34	21
28*	—	—	—	—	—	—	—	65	16
29	2	9	302	293	304	302	606	33	49
30	0	0	86	118	86	118	204	37	37
31	2	4	263	284	265	288	553	36	55
32	0	0	222	249	222	249	471	36	53
33	1	20	127	126	128	146	274	36	67
34	3	1	133	131	136	132	268	10	44
Total.....	23	84	2254	2350	2277	2434	4711	598	659
Average.....	—	—	—	—	—	—	428	40	44

* Sterile.

TABLE 4

Control for the high-producing inbred stock used in Experiment I, table 3; compare with table 2.

NO.	TOTAL NUMBER OFFSPRING	MALES	FEMALES	LIFE OF ♂	LIFE OF ♀
10	45	28	17	26+	26+
11	123	59	64	26±	26+
13*	—	—	—	35	26
14*	—	—	—	44	26
16*	—	—	—	40	15
17	311	159	152	40*	40
18	227	106	121	15	55
19	198	100	98	40	40
20	340	163	177	66	57
21	46	21	25	26	26
22	192	108	84	66+	34
23	231	115	116	66+	26
Total.....	1713	859	854	490	397
Average.....	190	—	—	41	33

* Sterile.

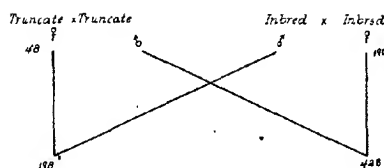


Diagram A Effect on productivity of crossing truncate and inbred stocks; summary from tables 3, 4 and 5.

producing strain, in fact, more than twice as many as when she was fertilized by her own male. The female of the low-producing strain was evidently producing a large number of eggs that were capable of fertilization as shown by the fact that on an average 198 of her eggs were fertilized. And yet the truncates used for control only produced an average of 48 to the fertile pair. The

TABLE 5

Control for the low-producing truncate stock, used in Experiment I, table 3; compare with table 1

NO.	LONG ♂	LONG ♀	TRUN- CATE ♂	TRUN- CATE ♀	TOTAL ♂	TOTAL ♀	GRAND TOTAL	LIFE OF ♂	LIFE OF ♀
1	2	1	9	13	11	14	25	25 ±	25 ±
2	1	5	6	4	7	9	16	30+	30+
3	6	9	59	83	65	92	157	30+	30+
4	1	0	33	40	34	40	74	23	30 ±
5*	—	—	—	—	—	—	—	34	34
6	0	0	17	19	17	19	36	37	14
7*	—	—	—	—	—	—	—	31	10
8	0	0	7	12	7	12	19	14	14
9	9	13	39	46	48	59	107	30 ±	30 ±
11	0	0	4	3	4	3	7	28 ±	23
12*	—	—	—	—	—	—	—	14	8
13	1	0	1	4	2	4	6	32	8
14	2	2	46	45	48	47	95	42	42
16*	—	—	—	—	—	—	—	32	32
17	0	1	18	10	18	11	29	37	42 ±
18	0	1	2	0	2	1	3	7	16
19*	—	—	—	—	—	—	—	33	19
20	7	0	25	13	32	13	45	27+	27+
21*	—	—	—	—	—	—	—	11	30
Total	29	32	266	292	295	324	619	517	464
Average...	—	—	—	—	—	—	48	27	25

* Sterile

crosses averaged even better than the high producing strain. Both were mutually benefitted by the cross, but it may seem that the production of the truncate female was limited because she lives on the average about half as long as the inbred female. The truncate male lives on the average about ten days longer,—long enough apparently to fertilize all the eggs of the inbred female. It should be stated that I have verified Castle's observation many times that more than one copulation is necessary to fertilize all of the output of eggs from a female. It seems that the male lives on the average long enough to fertilize this second output of eggs.

It is evident that the short life of the truncate female does not explain entirely her low production, for if the results of this experiment may be relied upon, there was evidently incompatibility of some sort between the egg and sperm in this strain.

The results of this experiment were very surprising in the light of Castle's work. Castle had made crosses between a low-producing strain and a high-producing strain with the result: "that low productiveness (or sterility) of the female may be transmitted directly through the egg from mother to daughter, but only indirectly through the sperm, the character skipping a generation." Castle's conclusion in regard to this case lends support to the assumption which I made in Part I, viz., that he did not distinguish between complete sterility of the female and the low fertility that is here shown.

The problem of the increased fertility of the truncate stock when crossed into a high producing strain was now put to another test. The experiment was carried out in the same way as the previous one except that the Woods Hole stock was used for the high-producing strain, as it had been observed in other experiments to give rise to a rather large number of offspring. Tables 6, 7 and 8 give the result of this experiment.

At first sight this experiment is not as striking an experiment as Experiment I. Neither cross did as well as would be expected from the controls. Nevertheless, the same relation is evident, for while the truncate female produced an average of 56 offspring by her own male, she produced on an average of 118 by the Woods Hole male. It is to be noted, moreover, that her length of life on

the average was only 15 days in the cross while in the control it was 25 days. It seems, not only from this, but from the other evidence, that had she lived on the average ten days longer her output would have been much greater than 118. The truncate male on the other hand was able to fertilize on an average at least 245 eggs of the female of the Woods Hole stock. But this length

TABLE 6a

Crosses between the low-producing truncate stock and the high-producing Woods Hole stock.

Truncate ♀ × Woods Hole ♂

NO.	CRES- CENT ♂	CRES- CENT ♀	LONG ♂	LONG ♀	TOTAL ♂	TOTAL ♀	GRAND TOTAL	LIFE OF ♂	LIFE OF ♀
1	11	13	152	169	163	182	345	25	24
2	8	3	28	64	36	67	103	19	13
3	1	0	49	61	50	61	111	15	13
4	2	0	111	108	113	108	221	23	14
5	3	1	20	31	23	32	55	?	4
6	2	0	15	25	17	25	42	29	14
7	1	0	16	19	17	19	36	34	13
8	1	0	28	36	29	36	65	15	28
9	2	0	42	48	44	48	92	23	17
10	3	2	43	62	46	64	110	38	11
Total....	34	19	504	623	538	642	1180	221	151
Average.	—	—	—	—	—	—	118	25	15

TABLE 6b

Reciprocal: Truncate ♂ × Woods Hole ♀

NO.	CRES- CENT ♂	CRES- CENT ♀	LONG ♂	LONG ♀	TOTAL ♂	TOTAL ♀	GRAND TOTAL	LIFE OF ♂	LIFE OF ♀
11	11	13	117	115	128	128	256	17	29
12	3	8	150	159	153	167	320	17	38
13	0	1	67	124	67	125	192	17	23
14	1	0	138	157	139	157	296	21	38
15	5	9	124	155	129	164	293	17	28
16	1	0	56	71	57	71	128	21	19
17	9	18	112	90	121	108	229	18	20
Total....	30	49	764	871	794	920	1714	128	192
Average.	—	—	—	—	—	—	245	18	28

of life is much shorter than in the controls. In other words, despite all of the unfavorable influences that entered into the crosses in comparison to the controls the point at issue is plainly evident that the female is producing a large percentage of eggs that are capable of fertilization, the male an abundance of good sperm, and yet when the two meet each other there is incompatibility of some sort between egg and sperm, as inferred by the number of offspring produced. It is clearly evident that the length of life is a factor, but by no means the only factor, involved in the low

TABLE 7

Control of Woods Hole stock. Brothers and sisters of the high-producing strain used in the cross in table 6

NO.	TOTAL NUMBER OFFSPRING	MALES	FEMALES	LIFE OF ♂	LIFE OF ♀
1	244	111	133	30	30
2	527	263	264	40	36
3	351	185	166	26	57
4	347	155	192	21	42
5	249	116	133	22	23
6	287	149	138	30	33
7	290	139	151	34	60
8	275	126	149	44	30
Total	2570	1244	1326	247	311
Average	321	155	166	31	39

TABLE 8

Control of truncate stock. Brothers and sisters of the low-producing strain used in the cross in table 6.

NO.	LONG ♂	LONG ♀	TRUN- CATE ♂	TRUN- CATE ♀	TOTAL ♂	TOTAL ♀	GRAND TOTAL	LIFE OF ♂	LIFE OF ♀
1	1	0	1	0		0	2	25	25
2	4	6	36	51	40	57	97	32	32
3	4	3	15	17	19	20	39	21	24
4	1	1	17	19	18	20	38	26	23
5	3	4	15	17	18	21	39	26	17
6	14	7	39	43	53	50	103	31	25
7	8	0	19	21	27	21	48	17	21
8	2	4	40	34	42	38	80	30	30
Total	37	25	182	202	219	227	446	208	197
Average	—	—	—	—	27	28	56	26	25

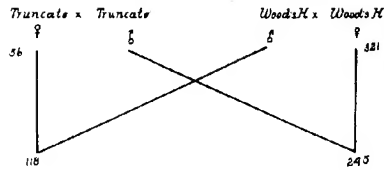


Diagram B Effect on productivity of crossing truncate and Woods Hole stocks; summary from tables 6, 7 and 8.

production of the truncates. To judge from the history of these strains and from Experiments I and II it would seem as though only one egg in four or five of the truncates gave rise to a mature fly when fertilized by its own sperm. I shall not analyze the data further here as I shall later bring forward better evidence that bears on this relation.

FERTILITY OF THE F_1 AND F_2 GENERATIONS FROM THE CROSSES
BETWEEN THE LOW-PRODUCING TRUNCATES AND THE HIGH-
PRODUCING WILD STOCKS AS DETERMINED BY BREED-
ING THE ANIMALS TOGETHER IN PAIRS

We may next consider the evidence that bears on the behavior of the hybrids in the F_1 and F_2 generations. In order to determine the productivity of the hybrids from the cross between the truncate female and the inbred male, 65 pairs of the F_1 brothers and sisters were made up from the crosses as given in table 3a. From the reciprocal cross 47 pairs of the F_1 brothers and sisters were paired. The different families were about equally represented. Both F_1 hybrids proved to be most virile animals and excellent producers. In the thirty days from December 22, 1911, when they were first paired as virgin flies, to January 22, 1912, when the experiment was discontinued due to an accident the sixty-five pairs produced a total of 16096, an average of 248 offspring to the pair, and the 47 pairs of reciprocals produced a total of 11800, an average of 251 offspring to the pair. The different classes that appeared are given in table 9.

The experiment shows that the hybrids are excellent producers and that it makes no difference whether the father or the mother was of the low-producing truncate stock. Moreover, it seems as

TABLE 9

Showing the number of offspring produced by 112 pairs of the hybrids in 30 days

	TRUN- CATE ♂	TRUN- CATE ♀	CRES- CENT ♀	CRES- CENT ♂	LONG ♂	LONG ♀	TOTAL ♂	TOTAL ♀	GRAND TOTAL	AVER- AGE
F ₁ (T♀ × I♂)...	516	778	431	633	6881	6837	7828	8268	16096	248
F ₁ (T♂ × I♀)...	273	332	315	436	4986	5458	5574	6226	11800	251

though the hybrids are long lived as shown by the fact that in this experiment at the end of thirty days 81 per cent of the flies were still living. Twenty-four males and 31 females had died. The death rate was not selective but was about equal in both the cross and its reciprocal.

Table 10 gives evidence on the same question— the output of the hybrids as tested in pairs. In this experiment the hybrids

TABLE 10

Showing production of the hybrids of the crosses between the truncate stock and the Woods Hole stock

TABLE 10 a						TABLE 10 b					
F ₁ generation from truncate ♀ × Woods Hole ♂						Reciprocal cross: F ₁ generation from truncate ♂ × Woods Hole ♀					
NO.	TOTAL ♂	TOTAL ♀	GRAND TOTAL	LIFE OF ♂	LIFE OF ♀	NO.	TOTAL ♂	TOTAL ♀	GRAND TOTAL	LIFE OF ♂	LIFE OF ♀
1	64	83	147	27	20	31	55	67	122	45	15
2	98	130	228	20	24+	33	92	124	216	57	24
4	96	119	215	27	33	34	92	105	197	40	24?
5	103	95	198	54	27	35	70	94	164	16+	24
8	143	169	312	24	35±	37	117	113	230	33	27
9	90	117	207	20	24	38	192	198	390	33	45
10	96	110	206	27	33	40	147	228	375	24	33+
11	100	140	240	45	24	41	99	129	228	37	15
13	53	67	120	20	15	42	54	72	126	24	27
14	167	138	305	40	27	43	104	113	217	12	24
16	101	139	240	40	33	44	106	127	233	40	20?
17	171	186	357	54	54	46	66	79	145	33+	27
18	142	150	292	63	20	47	17	23	40	20	12
19	87	100	187	33	49	49	219	269	488	24	63
20	126	146	272	27	33	50	112	126	238	45	—
22	238	244	482	39	19						
24	178	215	393	33	40	Total	1542	1907	3449	483	380
Total	2053	2348	4401	593	540	Av.			230	32	27
Av.			259	35	32						

between the truncates and the Woods Hole stock were used. The hybrids came from the cross given in table 6.

In the experiments given in table 11 the hybrids came from crosses between inbred and truncate stock, as recorded in tables 15a, b, Part I. At this time the inbred stock was in the F_1 generation and was a high-producing strain. The truncate stock was in the F_{12} generation.

The results of these three experiments make it certain that the hybrid offspring from the low-producing truncates and high producing wild stocks are high producers. In fact, the hybrids produce equally as well, if not better, than the high producing parental stock. Moreover, it makes little if any difference whether the father or mother is from the low-producing truncate stock. The following experiments give the evidence that bears on the question as to whether the low rate of fertility of one of the parents will reappear in the F_2 generation.

Tables 12 and 13 give the results of testing in pairs the offspring of the hybrids, derived from the crosses between the low-producing truncates and the high-producing wild stocks. The pairs as

TABLE 11

Hybrids of the crosses between the truncate stock and the inbred stock; the F_1 generation

TABLE 11a The F_1 generation from truncate ♀ × inbred ♂						TABLE 11b Reciprocal: The F_1 generation from truncate ♂ × inbred ♀					
NO.	MALES	FE- MALES	TOTAL	LIFE OF ♂	LIFE OF ♀	NO.	MALES	FE- MALES	TOTAL	LIFE OF ♂	LIFE OF ♀
7	294	295	589	42	39	1	184	256	440	58	48
8	180	224	404	56	41	6	174	213	387	60	40
16	197	228	425	23	55	9*	91	119	210	16	16
22	164	176	340	40	45	12	120	170	290	80	40
32	235	215	450	39	33	19	121	137	258	73	23
36	50	6	111	25	61	Total	690	895	1585	287	167
10	244	246	490	23	73	Av.	.	.	317	57	33
11	259	242	501	45	62						
30	166	208	374	58	44						
Total	1789	1895	3684	351	453						
Av.			409	39	50						

* Died at end of sixteen days because of failure to feed on transfer to new bottle.

given in table 12 came from the hybrids recorded in table 9. The pairs given in table 13a, b came from the hybrids recorded in table 11.

Tables 12 and 13 appear to show that low production reappears after skipping a generation and that this low production is transmitted through both the egg and sperm to the grandchildren. I shall not attempt to analyze the data further as I have other and more exact evidence that bears on the transmission of the low fertility of the truncates.

CROSSES BETWEEN THE LOW-PRODUCING TRUNCATES AND THE
HIGH-PRODUCING WILD STOCKS IN WHICH AN EXACT
MEASURE OF FERTILITY IS EMPLOYED

The term 'fertility' is used in so many different ways by different writers that I wish to make clear at the outset the sense in which the term is used throughout the ensuing papers. By 'fertility,' I mean the percentage of eggs that complete development and give rise to mature flies. For example, if 100 eggs are isolated from a stock and later 50 flies come from these eggs then I speak of the fertility of that stock as 50 per cent. It is evident that fertility in this sense can only be determined by isolating the eggs, and that the number of offspring produced by a pair does not give a measure of the fertility of that pair but only a measure of the 'productivity' of that pair.

The marked difference in the length of life between the truncate stock and the wild strains together with the increased length of life of the hybrid makes it very difficult to draw safe conclusions in regard to the rôle played by the egg and sperm in fertilization in so far as establishing any definite ratios are concerned. In other words the production of offspring cannot be taken as an absolute measure of the fertilizing power of the egg and sperm for as we shall see later in the case of the hybrids, although their production of offspring is very high, their fertility (combination of egg and sperm) is relatively low.

Accordingly, I wish to return to the question of the low fertility of the truncates and consider the behavior of the low fertility in this race on crossing into other races. In order to insure an

TABLE 12

Showing the result of mating in pairs the F_2 generation of the crosses between the short-lived, low-producing truncates and the relatively long-lived high-producing inbred stock

TABLE 12 a

Grandchildren of the cross between the truncate φ and inbred σ^7

FAMILY NUMBER OF GRANDPARENTS	FAMILY NO. P ₁	NUMBER	NO. OFFSPRING PRODUCED	LIFE OF σ^7	LIFE OF φ
5	34	113	0	24	7
	33	137	5	15	7
	33	147	49	17	36
	34	157	331	38+	38+
	34	161	319	24	29
	33	167	159	14	35
	33	172	106	47	?
7	33	173	330	32	46
	91	118	0	—	—
	91	122	0	—	—
	91	125	0	—	—
12	91	155	0	—	—
	107	114	111	24	21
	107	116	42	42	14
	106	121	50	?	14
	106	123	251	31	31
	50	129	25	12	?
	106	130	29	32	7
	50	134	280	20	39
	107	135	62	9	23
	50	138	164	30	38
	50	146	208	25+	25
	106	150	224	23	30
	106	154	75	20	15
	106	175	178	29	35
	107	182	0	—	—
	107	184	44	21	12
14	117	112	196	26	48
	117	115	0	—	—
	23	139	67	39	?
	26	143	171	36	46
	26	145	0	—	—
	117	158	0	—	—
	117	159	21	55	?
	117	162	8	16	12
	26	180	204	59	46
	26	184	44	21	12
16	80	126	190	35+	35+
	80	151	288	37	32
	14	26	183	15	51
Av.			140	30-	30-

TABLE 12 b

Reciprocal cross: Grandchildren of the cross between the truncate σ^7 and the inbred φ

FAMILY NUMBER OF GRANDPARENTS	FAMILY NO. P ₁	NUMBER	NO. OFFSPRING PRODUCED	LIFE OF σ^7	LIFE OF φ
20	111	119	74	14+	14+
	111	120	0	—	—
	111	140	256	38	46+
	111	149	0	—	—
	111	153	150	41	44
	111	163	152	5	33
	111	165	0	—	—
	111	178	215	35+	35+
	111	181	135	16	16
	120	102	111	89	40
29	95	117	153	24+	24+
	102	124	192	14	24
	100	127	136	18+	34
	102	128	98	7	7
	95	131	200	34	34
	95	132	200	26	26
	102	133	107	20	23
	100	136	6	38	6
	102	141	129	27	23
	102	142	8	41	6
	102	144	42	39	15
	95	152	46	6	39
	81	156	191	33	?
	102	160	298	38	?
	100	161	432	32	32
	102	166	379	24	35
	102	168	61	22	22
	102	169	189	29	24
	102	170	288	?	32
30	95	171	226	38	26
	102	174	111	24	26
	95	176	205	54	26
	102	177	43	19	22
	102	179	230	38	24
	102	185	425	32	37
	113	148	0	—	—
Av.			171	30-	26-

TABLE 13a

Table showing result testing in pairs the grandchildren of the crosses between the short-lived low-producing truncates and the relatively long-lived, high-producing inbred stock. T = truncate; C = crescent

Grandchildren of the cross between the truncate ♀ and inbred ♂

FAMILY NO. OF GRAND- PARENTS	FAMILY NO. P ₁	NO.	TOTAL ♂	TOTAL ♀	GRAND TOTAL	LIFE OF ♂	LIFE OF ♀	
33	7	415	29	41	70	40	16	
		416	18	33	51	45	16	
		417 C	23	6	29	52	19	
		418	0	0	0	32	6	
	8	386	34	37	71	27	27	
		387	119	182	301	49	33	
		388	21	41	62	15	15	
		16	389 T	0	0	0	16	30
	390		0	0	0	46	44	
	391 C ♂		67	72	139	27	27	
	392 C ♂		73	113	186	39	26	
	34	32	393	43	37	80	16	16
			394	92	102	194	60	17
			395	77	121	198	61	25
			436 T	29	25	54	15	15
		22	437	0	0	0	54	15
			438 T	18	20	38	54	11
			432	77	79	156	52	16
			433 C	145	131	276	52	29
		35	36	397	103	113	216	30
398				71	81	152	53	33
399				68	72	140	39	20
407				60	74	134	63	27
10	408		98	102	200	16	16	
	414		20	21	41	16	21	
	400		151	134	285	34	56	
	401		83	69	152	45	31	
35	11	402 T ♂	88	127	215	9	37	
		403 T	78	73	151	31	27	
		427	51	63	114	16	20	
		428	74	100	174	22	32	
	30	429	71	67	138	?	42	
		430	62	47	109	40	18	
		410	2	2	4	37	56	
		434	14	14	28	16	6	
		435	0	0	0	50	6	
		Total.....		—	1959	2229	4188	1279
Average...		—	—	—	135	36	25	

exact measure of the fertilizing power of the egg and sperm the experiments were carried out in the following manner.

A female was placed in a bottle with four or five males to insure an abundance of sperm. A small piece of banana was introduced on a piece of paper and on this food the female laid her eggs. Each day the food was removed and the eggs picked off individually with the point of a needle. Each batch of eggs was planted on fresh food and the number of eggs recorded. After a few hours new food was given to the parents. This is a most laborious process so that one cannot carry out as many crosses at a time as might be desired, but this does not invalidate the test as such.

Since the Woods Hole stock was the most fertile one at hand, six females were tested with their brothers as a control. Attention is called to the fact that these females were about ten days old when the experiment began. Eleven truncate females were tested against the Woods Hole male. Fourteen truncate females were tested against their truncate brothers. These counts have led to the results given in detail in table 14, a, b and c.

TABLE 13 b

Reciprocal cross: Grandchildren of the cross between the truncate ♂ and the inbred ♀.

FAMILY NO. OF GRAND- PARENTS	FAMILY NO. P ₁	NO.	TOTAL ♂	TOTAL ♀	GRAND TOTAL	LIFE OF ♂	LIFE OF ♀
25	1	420 T	74	71	145	32	45
		421	40	59	99	19	29
		422	0	1	1	20	20
		423	0	0	0	52	26
	6	424	99	104	203	20	42
		425	51	39	90	52	16
		426	92	92	184	22	45
	12	411	7	0	7	55	11
		412	64	68	132	34	29
		413	17	20	37	50	22
		419	0	0	0	11	52
		404	22	28	60	15	15
		405	82	90	172	62	52
		431	21	36	57	29	52
Total....	—	—	569	518	1087	472	436
Average..	—	—	—	—	91	34	31

TABLE 14

Egg count showing fertility of the truncates stock bred to the Woods Hole stock

TABLE 14a

Woods Hole ♀ × Woods Hole ♂

NO.	NO. DAYS COUNTED	TOTAL NO. EGGS ISOLATED	NO. NOT CONSIDERED	NO. CONSIDERED	NO. FLIES EMERGED
1	27	227	0	227	148
2	28	302	0	302	256
3	5	40	0	40	30
4	25	424	0	424	320
5	3	20	0	20	10
6	24	389	0	389	320
Total	—	1402	0	1402	1084

TABLE 14b

Truncate ♀ × truncate ♂

NO.	LIFE OF ♀	TOTAL NO. EGGS LAID	NO. NOT CONSIDERED	NO. CONSIDERED	NO. FLIES EMERGED
18	30	271	120	151	42
19*	23	268	177	91	19
20	28	220	148	72	6
21	5	12	2	0	0
22	36	239	0	239	56
23	57	560	111	449	76
24	42	435	32	403	89
25	32	304	23	281	87
26*	38	360	0	360	86
27	20	295	0	295	69
28	34	282	0	282	114
29	6	13	13	0	0
30	5	0	0	0	0
31†	42	394	0	394	150
Total	—	3249	626	2623	644

TABLE 14c

Truncate ♀ × Woods Hole ♂

NO.	LIFE OF ♀	TOTAL NO. EGGS LAID	NO. NOT CONSIDERED	NO. CONSIDERED	NO. FLIES EMERGED
7	5	17	0	17	8
8	8	93	0	93	58
9	9	31	24	7	6
10	10	20	20	0	0
11	3	2	2	0	0
12	28	226	0	226	148
13	14	179	0	179	68
14	6	1	0	1	1
15	9	21	0	21	8
16*	6	6	6	0	0
17	7	18	3	15	10
Total	—	614	55	559	307

* Escaped.

† This was a long winged female and is not considered in the totals. Note that her fertility was somewhat higher than the average. This will be discussed later.

In this experiment the entire output of eggs of the truncates was collected. The count from the Woods Hole stock does not represent the entire output since these flies were about ten days old when the experiment began. It is true that it is practically impossible to get the entire output of eggs by relying upon an ordinary dissecting microscope without spending an undue amount of time, but as the same method was applied throughout this source of error as it enters into a comparison of the output of eggs of the different races is about constant. I feel quite sure that the counts, as here shown, represent over 80 per cent of all the eggs laid. That many were missed is certain as the food from which the eggs were picked was often kept and later larvae were seen to emerge. In regard to the conditions of the experiment as a test of viability, no objection on the grounds of personal selection can be made, as it is impossible to tell beforehand which eggs are going to hatch. Care must be taken not to allow the eggs to stand too long before picking them as the larva will emerge from the fertile ones while the sterile eggs are left behind to be picked up and considered in the counts.

The question may arise in the mind of the reader as to why 626 eggs are not considered in table 14 b in determining the percentage of eggs which hatched. The explanation is given here since the question recurs in some of the other tables and in succeeding experiments. It is evident from the method used in making the test that a picture is given of the productivity of any single female day by day. The 120 recorded in column "No. not considered"—No. 18 (table 14 b) represents the total number of eggs laid by the female through a number of consecutive days in which none of her eggs hatched. The eggs that failed to hatch were in practically all cases those isolated during the first few days of the experiment, although in a few cases they came in the middle of the count, more frequently however toward the close of the count. This seems a fair way to treat the facts as this may be due to other causes operating other than eggs meeting sperm.

In this case the benefit of the doubt is given to the truncates for if these are considered 749 in 3643, or 21 per cent, hatched. In reality the percentage is not changed much and had this been

known in the beginning much labor could have been saved by picking the eggs from mass cultures, although this would give little insight into the total output of eggs from a single female. The former plan allows us to get much closer to the problem in hand. The results of Experiment IV are given in diagram C.

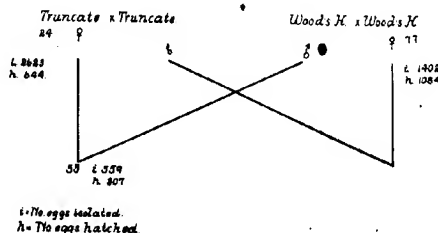


Diagram C Effect on fertility of crossing truncate and Woods Hole stocks; from table 15.

This experiment served in a preliminary way to show that the egg-picking process could be relied upon in general as it gives results in harmony with the earlier breeding experiments. The evidence seems to show conclusively that only one egg in four or five of the truncate female, when fertilized by its own male, will complete development. Moreover, it is shown that the Woods Hole male can fertilize more than twice as many of the truncate eggs than can her own male although it has been shown in earlier experiments that he produces an abundance of fertile sperm. It is interesting to note that only 77 per cent of the eggs of the Woods Hole stock hatched. It was the expectation that this stock would show a fertility of something like 100 per cent. At first I was inclined to believe that this discrepancy was due to some defect in the egg picking process itself, but the results of later experiments together with the behavior of this stock does not bear out any such conclusion. Since these experiments were completed I have obtained a fertility of 96 per cent with two new wild stocks.

It seemed to me that the explanation for the low production of the truncates was not far to seek. That the length of life is a factor is not to be doubted, but more than that it is evident from

the experiments that only relatively few eggs were giving rise to mature flies. I wished to test the conclusion further and also determine whether or not some factor for egg production was operating, as well as to make the cross between the truncate male and the Woods Hole female which combination was not made in the previous experiment.

Experiment V. This experiment was carried out in the same manner as the former one. The flies were mated as soon as they emerged and the output of eggs recorded from each female each day during her life. The facts in detail are recorded in table 15.

The results of this experiment are expressed in diagram D.

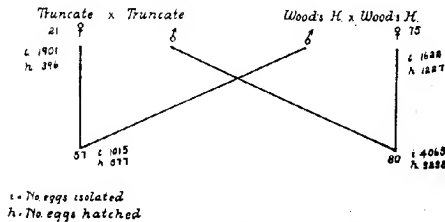


Diagram D. Effect on fertility of crossing truncate and Woods Hole stocks; from table 16.

This evidence taken with all the other evidence presented shows conclusively that there is incompatibility between the egg and sperm of the truncates since only one egg in every four or five gives rise to adult flies. It is surprising to find that the truncate male should have actually fertilized more eggs of the female of the Woods Hole stock than a male from its own stock could fertilize, and yet a male from the Woods Hole stock can fertilize almost as many eggs of the truncate female as he can fertilize with a female of his own race. If the results given in diagram D are compared with those obtained in Experiment I as expressed in diagram A; it will be found that the results are in general agreement.

Experiment VI. Reference to table 2, where the history of the inbred stock is given, will show that the fertility has steadily declined on inbreeding, despite the fact that sterile females had increased to 50 per cent and again eliminated. It occurred to me that something like incompatibility between egg and sperm might be operating in this stock

TABLE 15

Egg^{*} count showing the fertility of the truncate stock together with the fertility on crossing into the Woods Hole stock

TABLE 15 a

Woods Hole ♀ × Woods Hole ♂

NO.	LIFE OF ♀	TOTAL NO. EGGS LAID	NO. NOT CONSIDERED	NO. CONSIDERED	NO. FLIES EMERGED
24	52	463	0	463	356
25*	51	650	194	456	138
26	33	316	0	316	148
27	52	529	0	529	433
28	25	314	0	314	290
Total	—	2272	194	2078	1365

* Laid very small eggs; some looked as if they might have been parasitized.

TABLE 15 c

Truncate ♀ × Woods Hole ♂

NO.	LIFE OF ♀	TOTAL NO. EGGS LAID	NO. NOT CONSIDERED	NO. CONSIDERED	NO. FLIES EMERGED
14	14	33	18	15	4
15*	18	0	0	0	0
16	4	0	0	0	0
17	19	265	0	265	214
18†	6	84	0	84	64
19	12	45	39	6	3
20	22	259	6	253	132
21	30	235	53	182	62
22	16	105	0	105	42
23	18	133	28	105	56
Total	—	1159	144	1015	577

* Escaped.

† Copulated.

TABLE 15 b

Truncate ♀ × Truncate ♂

NO.	LIFE OF ♀	TOTAL NO. EGGS LAID	NO. NOT CONSIDERED	NO. CONSIDERED	NO. FLIES EMERGED
1	32	359	19	340	90
2	12	2	2	0	0
3	23	251	220	31	7
4	24	47	14	33	10
5	4	21	21	0	0
6	35	374	0	374	64
7	7	23	23	0	0
8	6	74	0	74	21
9	39	567	27	540	148
10	49	282	96	186	25
11	32	320	0	320	30
12	6	22	22	0	0
13	19	45	42	3	1
Total	—	2387	486	1901	396

TABLE 15 d

Woods Hole ♀ × Truncate ♂

NO.	LIFE OF ♀	TOTAL NO. EGGS LAID	NO. NOT CONSIDERED	NO. CONSIDERED	NO. FLIES EMERGED
29	72	730	0	730	617
30	22	323	0	323	276
31*	31	631	0	631	527
32	78	536	17	519	395
33	79	552	18	534	389
34	75	798	12	786	588
35	67	557	15	542	446
Total	—	4127	62	4065	3238

* Escaped.

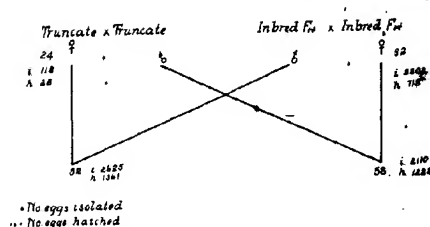


Diagram E Showing the effect on fertility of crossing the inbred and truncate stocks.

and if so this could be put to a test and at the same time more light might be thrown upon the behavior of the truncate stock. Accordingly the inbred stock which was now in the F_{14} generation was crossed into the truncate stock. Table 16 gives the details of the experiment. Table 17 is the control on the inbred stock that was bred in pairs at the same time.

The results of Experiment VI are expressed in diagram E.

BACK CROSSES BETWEEN THE HYBRIDS AND THE RECESSIVE LOW-PRODUCING TRUNCATES

We may next consider the evidence that bears on the fertility of the hybrids when paired with each other and the fertility of the hybrids when back crossed into the recessive low-producing truncates. This experiment was originally undertaken in order to determine whether or not there were sex-linked factors for fertility. The results in this direction have been largely negative but nevertheless the experiments give us the key by which the low productivity of the truncates may be explained on a very simple assumption.

It was my expectation to find that the eggs of the hybrids when isolated would show a fertility of 100 per cent, since I had already found that their production is far in excess of their high-producing parent. Three experiments have been required in order to convince myself that the fertility of the hybrids is in reality low and that the percentage of fertility in the back crosses is an actual measure of the fertilizing power of the egg and sperm.

There are two classes of hybrids, as follows: Offspring from the truncate ♀ by Woods Hole ♂ will be referred to as A ♀ and A ♂.

TABLE 16

Egg count showing the fertility of the inbred stock together with the effect on fertility of crossing into the truncate stock

TABLE 16a
Inbred ♀ × inbred ♂

NO.	LIFE OF ♀	TOTAL NO. EGGS LAID	NO. NOT CONSIDERED	NO. CONSIDERED	NO. FLIES EMERGED
1	28	340	0	340	89
2	21	175	21	154	60
3*	15	325	0	325	68
4	36	400	30	370	116
5	35	403	0	403	174
6†	35	453	20	433	140
7	19	137	3	134	67
8	23	180	137	43	4
Total	—	2413	211	2202	718

* Lived much longer but ceased to lay eggs after 15 days; preserved for study.

† Discontinued.

TABLE 16c
Inbred ♀ × truncate ♂

NO.	NO. DAYS COUNTED	TOTAL NO. EGGS LAID	NO. NOT CONSIDERED	NO. CONSIDERED	NO. FLIES EMERGED
25*	28	291	55	236	111
26*	28	166	12	154	29
27*	26	303	30	273	154
28	27	278	0	278	193
29*	32	107	47	60	18
30*	22	375	0	375	263
31*	22	407	0	407	225
32*	22	327	0	327	229
Total	—	2254	144	2110	1222

* Discontinued.

TABLE 16b
Truncate ♀ × Inbred ♂

NO.	LIFE OF ♀	TOTAL NO. EGGS LAID	NO. NOT CONSIDERED	NO. CONSIDERED	NO. FLIES EMERGED
9	4	0	0	0	0
10	14	18	14	4	1
11	20	221	33	188	88
12	17	112	18	94	45
13	4	0	0	0	0
14	6	0	0	0	0
15	20	101	3	98	38
16	10	130	15	115	38
17	15	122	34	88	46
18	11	96	9	87	39
19	3	0	0	0	0
20	5	45	5	40	32
21*	17	0	0	0	0
22	27	268	0	268	164
23	9	26	12	14	4
24	20	126	14	112	29
33	17	128	0	128	83
34	13	139	5	134	75
37	11	106	0	106	43
38	19	253	0	253	159
39	5	0	0	0	0
40	10	0	0	0	0
41**	17	256	0	256	137
42**	17	251	50	201	123
43	15	126	11	115	41
44†	9	0	0	0	0
45**	17	184	19	165	86
46†	9	0	0	0	0
47	17	72	10	62	39
48††	12	0	0	0	0*
49	15	97	0	97	51
Total	—	2877	252	2625	1361

* Escaped.

** Discontinued.

† Killed and preserved for study.

†† Laid a few cheesy looking eggs probably due to parasites.

TABLE 16d
Truncate ♀ × truncate ♂

NO.	LIFE OF ♀	TOTAL NO. EGGS Laid	NO. NOT CONSIDERED	NO. CONSIDERED	NO. FLIES EMERGED
35	26	179	70	109	26
36	8	9	0	9	2
Total	—	188	70	118	28

Offspring from the reciprocal cross will be referred to as B ♀ and B ♂. In these experiments A ♀ is tested with A ♂, B ♀ with B ♂, A ♀ with the truncate ♂, B ♀ with the truncate ♂, the truncate ♀ with A ♂ and the truncate ♀ with B ♂. In order to get an exact measure of the fertility, eggs were isolated day by day from each of the combinations made. The number of eggs isolated each day, and the number of flies that emerged is given in table 18.

TABLE 17

F₁₄ generation of the inbred stock, showing the result of testing the flies in pairs; compare with table 16a

NO.	NO. OF ♂	NO. OF ♀	TOTAL	LIFE OF ♂	LIFE OF ♀
1	59	67	126	43	26
2	55	39	94	31	31
3	113	133	246	45	37
4	33	31	64	41	10
5	0	0	0	91	13
6	89	90	179	77	21
7	129	139	268	37	37
8	70	90	160	81	17
9	129	133	262	41	35
10	28	31	59	30	24
11	104	111	215	30	28
12	46	46	92	28	24
13	128	96	224	39	28
14	71	59	130	37	25
15	44	77	121	28	28
Total.....	1098	1142	2240	679	384
Average.....	—	—	159	45	26

The percentage of eggs that hatched in this experiment is not altogether reliable, due to unfavorable conditions which arose. Previous to this time the flies had hatched normally and I still believe that the foregoing experiments measure the absolute fertility of the different stocks and combinations made, within a range of 3 per cent. In this experiment, however, the food became very sour and the flies did little more than crawl out of the pupae and die. The dead animals were searched for and counted. The percentages as given in this table are in consequence too low and are to be taken relatively to each other and should not be compared with the percentages as given in the other tables. Tables 19 and 20 give the details of a second and third repetition of this experiment. The results express more accurately the facts in regard to fertility in these combinations. The plan of the third experiment, table 20, was modified somewhat for it seemed that fertility could be determined fairly accurately by isolating the eggs from mass cultures provided the experiment were controlled by a stock the fertility of which was known. Accordingly hybrids between the truncates whose fertility was low and the Woods Hole stock whose fertility was high were produced and a number of these hybrids were tested in mass culture. The hybrids were also back crossed into the truncates as shown in table 20. In each case a number (the actual number is indicated in the table) of virgin females was placed in a bottle and twice as many males from the stock, in which the combination was desired, added. The males and females were kept separate for four or five days before they were placed together.

At the time of placing the two sexes together an epidemic of mating took place. In each of the bottles about half the pairs were to be seen mating at the same time. None of the eggs were isolated until five days later in order to give all the females a chance to mate and in order to avoid the first few eggs laid, which as I have shown, are not likely to hatch, especially in the case of the truncates. The eggs were isolated day by day in the usual manner. Table 20 gives the result of this experiment. It will be noticed that it is in close agreement with the second experiment of this series.

TABLE 19

Showing the combinations made between the hybrids and the truncates; the number of eggs isolated and the corresponding number of eggs that hatched

T ♀ × B ♂				
NO.	NO. DAYS COUNTED	NO. EGGS ISOLATED	NO. FLIES HATCHED	EGGS NOT INCLUDED
13	2	0	0	0
14	4	35	19	14
15	13	200	69	0
16	2	0	0	0
17	3	40	14	0
18	3	24	8	0
19	4	39	20	0
20	4	45	8	2
21	6	20	7	0
22	14	104	44	12
23	14	31	10	25
24	13	144	38	0
25	9	125	48	5
26	7	120	39	11
27	14	0	0	9
28	20	0	0	0
Total		927	324	78

35 per cent

B ♀ × B ♂				
NO.	NO. DAYS COUNTED	NO. EGGS ISOLATED	NO. FLIES HATCHED	EGGS NOT INCLUDED
6	2	28	22	0
7	16	413	220	0
8	3	75	52	0
9	14	257	165	0
10	6	230	129	0
11	5	134	72	0
12	20	437	277	0
Total		1574	937	0

59.6 per cent

A ♀ × A ♂				
NO.	NO. DAYS COUNTED	NO. EGGS ISOLATED	NO. FLIES HATCHED	EGGS NOT INCLUDED
1	20	460	278	0
2	20	392	164	0
3	5	144	79	0
4	3	57	30	0
5	20	382	257	0
Total		1435	808	0

56.3 per cent

A ♀ × T ♂				
NO.	NO. DAYS COUNTED	NO. EGGS ISOLATED	NO. FLIES HATCHED	EGGS NOT INCLUDED
39	18	284	122	0
40	13	218	107	0
41	20	585	368	0
42	17	414	287	0
43	20	353	210	0
44	20	296	125	136
45	20	283	103	0
Total		2433	1322	136

54.3 per cent

B ♀ × T ♂				
NO.	NO. DAYS COUNTED	NO. EGGS ISOLATED	NO. FLIES HATCHED	EGGS NOT INCLUDED
46	20	513	321	0
47	11	296	158	0
48	20	400	209	11
49	20	481	179	13
50	20	481	232	0
Total		2171	1099	24

50.6 per cent

T ♀ × A ♂				
NO.	NO. DAYS COUNTED	NO. EGGS ISOLATED	NO. FLIES HATCHED	EGGS NOT INCLUDED
29	20	276	150	0
30	8	110	41	0
31	7	103	39	0
32	13	16	5	25
33	3	55	22	0
34	17	117	51	0
35	3	0	0	0
36	10	50	10	0
37	20	141	64	0
38	20	181	76	0
Total		1049	458	25

43.4 per cent

Diagram F expresses the essential relation of fertility as brought out in these three experiments. I shall consider the results of the last two experiments in making up the average since it is evident that these numbers express more nearly the actual facts of the experiment.

TABLE 20

Back crosses between hybrids (of Woods Hole and truncate stock) and the truncate stock. The table gives the number of females that entered into each cross. The numbers on the left side of each column show the number of eggs isolated, the corresponding number on the right the number of flies that emerged.

CROSS		A ♀ × T ♂	B ♀ × T ♂	T ♀ × A ♂	T ♀ × B ♂	B ♀ × B ♂	A ♀ × A ♂	W ♀ × W ♂
NO. FEMALES		12	15	28	28	15	15	15
		eggs adults	eggs adults	eggs adults	eggs adults	eggs adults	eggs adults	eggs adults
Jan. 23 '13		110-45	120-65	150-39	61-7	217-130	180-111	225-130
24		60-20	114-54	70-26	43-13	100-0*	103-7*	95-45
25		161-89	130-63	74-7*	55-18	131-72	160-43*	116-72
26		135-41	142-82	120-39	150-56	151-87	115-47	150-97
27		103-47	90-29	133-21	170-40	151-100	150-48	140-91
28		105-39	150-55	200-86	160-74	165-104	166-73	175-144
29		160-80	200-119	175-63	146-42	190-145	231-153	176-123
30		110-33	88-73	125-58	107-46	112-86	203-135	120-81
31		45-25	63-44	61-49	51-16	70-50	100-65	95-67
Feb. 1		71-35	106-55	94-40	45-11	61-45	125-94	100-75
2		90-53	86-49	80-52	30-10	110-80	125-89	36-29
3		40-28	24-14	33-18	20-6	59-41	42-29*	25-17
4		38-20	45-29	53-27	43-19	35-25	60-39	44-31
5		20-8	45-22	50-18	10-3	35-25	21-12	30-21
6		43-23	50-24	34-18	20-7	35-31	86-59	
7		27-12	78-37	45-15	30-8	100-62	130-58	98-76
8		39-16	91-44	34-12	27-8	102-55	120-55	50-38
9		52-25	150-75	105-40	43-12	53-34	100-49	100-74
10		160-88	85-49	100-34	28-0*	121-63	150-80	66-50
11		140-73	114-42	102-36	17-1	115-70	115-60	135-81
13		50-33	51-22	50-20	0	55-28	53-38	54-36
14		51-26	50-18	50-17	0	55-30	56-30	55-43
Total		1810-859	2072-1064	1864-728	1228-397	2122-1363	2328-1324	2085-1421
Per cent. . . .		47	51	39	32	64	57	70

* These bottles met with an accident and are not considered in the totals.

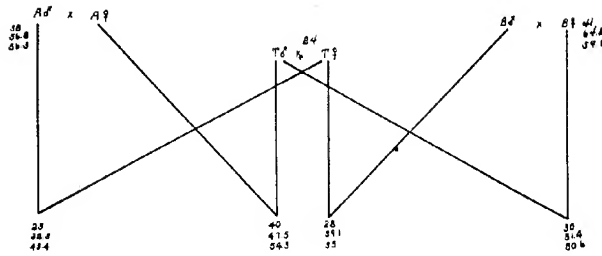


Diagram F Summary of tables 18, 19 and 20, showing the effect on fertility of back-crossing the hybrids into the recessive low-producing truncates.

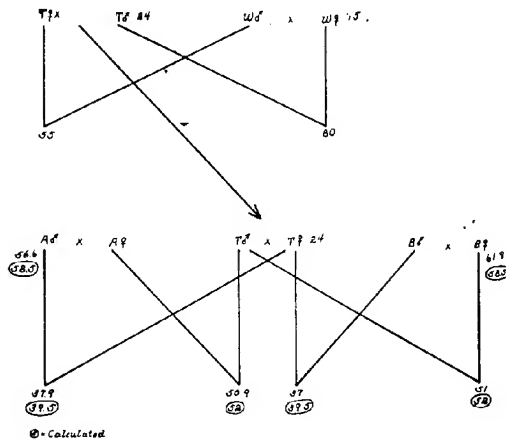


Diagram G Expresses the essential relations of the behavior of fertility in heredity as based on the foregoing experiments. The numbers in the circle express the calculated percentage. The other numbers express the observed percentage of fertility.

In diagram G a composite picture is given of all the experiments that bear on fertility in these crosses where an accurate method of measurement has been employed.

If 24, 75, 55, and 80 express the actual fertilizing powers of the gametes of the different combinations then the percentages as given in the hybrid back crosses can be explained on the following assumption.

The hybrid A is made up of T ♀ and W ♂. Let us assume that its germ cells segregate into the parental types in so far as factors for fertility are concerned. Accordingly A ♀ produces two kinds of eggs, T eggs and W eggs. A ♂ will produce two kinds of sperm, T sperm and W sperm. Diagram H expresses the relation as to what happens when the gametes are brought together.

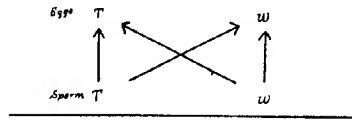


Diagram H Showing the possible combinations of the gametes of the hybrids (A) on the assumption that the germ cells have segregated into the parental types in respect to factors for fertility.

When a T sperm fertilizes a T egg 24 per cent of the eggs hatch
 When a T sperm fertilizes a W egg 80 per cent of the eggs hatch
 When a W sperm fertilizes a T egg 55 per cent of the eggs hatch
 When a W sperm fertilizes a W egg 75 per cent of the eggs hatch

The sum, 234, divided by 4 gives on this assumption the actual fertilizing power of the combination, or 58.5, which corresponds very closely with the 56.6 observed. In like manner the other combinations may be determined. I have placed in a circle in each case the calculated percentage of fertility; the number outside the circle expresses the observed percentage. The agreement between the two sets of numbers lends support to the view that the germ cells of the hybrid do segregate into factors identical with those of the parents in so far as factors that are responsible for fertility are concerned.

We find here, I believe, the key for the low fertility of the truncates. They have become homozygous for many factors. The corresponding rise in fertility when crossed into other races is to be explained on the assumption that the combination formed is heterozygous for more characters upon which fertility depends and consequently more likely to develop. The assumption seems a reasonable one to make in light of the fact that we are able to predict the fertilizing power of the gametes in a given combination.

It will be recalled that over sixty generations of continuous selection has failed to produce pure truncate stock. May not this result be explained on the grounds that only the heterozygous forms with respect to this structure ever reach maturity; the homozygous or pure truncate animals never come into existence? It is of interest in this connection to recall Cuénot's failure to produce yellow mice that would breed true.

The reduced fertility of the inbred stock in successive generations of inbreeding may also find its explanation on the assumption that the gametes have reduced to a condition approaching homozygosity for as I was able to show in the F_{11} generation a larger percentage of zygotes developed as a result of outbreeding.

FERTILITY AND HIGH PRODUCTIVITY OF THE HYBRIDS

It has been pointed out that the hybrids that resulted from the crosses between the low-producing truncates and the high-producing wild stocks give rise to a large number of offspring. They produce on an average many more young than their high-producing parents. The fertilizing power of their gametes when inbred was only 56.6 per cent, while in the Woods Hole stock it was 75 per cent. In other words, the productivity of the hybrids is higher than its high-producing parent, but the fertilizing power of its gametes is lower.

This apparent contradiction is accounted for by the fact that the output of eggs of the hybrid female is greatly increased and despite the fact that the fertilizing powers of the egg and sperm when placed together is relatively low, nevertheless the production of offspring is very high. This fact shows how easily one might be misled by using the number of offspring as a measure of the fertilizing power of the egg and sperm when not controlled by more accurate means. A comparison of the number of eggs laid by the hybrid, table 21, with the number laid by the Woods Hole females, tables 14 a, 15 a and 15 d will show that the output of eggs from the hybrid is practically twice that of its high-producing parent.

The following controlled experiment, table 22, confirms the foregoing conclusion and also shows that it makes no difference

in regard to egg production whether the father or the mother of the hybrid came from the low-producing truncate stock.

In this experiment conditions were as uniform as possible. The virgin females were in each case paired with three of their brothers. The eggs were counted each day with the aid of a dissecting microscope and discarded. The females in this experiment were given a chance to lay their eggs on dried apples that had been soaked in water and allowed to become slightly sour. This insures great accuracy in counting, as the white eggs are easily seen against a brown background.

The count as given in tables 21 and 22 supports the conclusion that the hybrid female has a greatly increased output of eggs as compared with the Woods Hole female. Now despite the fact that only about 56 per cent of her eggs hatch when tested with her own male, yet she is able to produce as many if not more offspring than her high producing parent, the fertility of which is 75 per cent. We should expect the hybrid to lay approximately twice as many eggs. An analysis of table 22 shows this to be true. It is true that no far-reaching conclusion can be based on such a round-about treatment as this, taken alone. This evidence must be taken in connection with the other evidence presented. If the percentage evidence as given is correct, and if it is true that the hybrids are excellent producers, as judged by the number of offspring produced, then we should expect the egg-production to be greatly increased. The count as given here verifies the expectation and makes the former conclusions more certain.

The anomalous condition that the fertility of this animal may be relatively low and yet its productivity high, finds its explanation in the facts presented and shows, as before stated, how one may easily be misled in regard to fertility in heredity by using the number of offspring produced as a guide, when not checked up by more accurate means.

The increased egg-laying capacity on the part of the female may seem strange at first sight, especially when we recall the fact that neither parent possessed it to such a marked degree. But let it be recalled that the hybrid is more virile, as judged by the length of life, than either parent and that the same thing is probably true in regard to their reaction to light. In fact, in every way these

TABLE 21

Number of eggs laid by the offspring of the crosses between the low-producing truncates and the high-producing Woods Hole stock. These are the same females given in table 18.

(A) A ♀ × A ♂			(B) B ♀ × B ♂		
NO.	DAYS COUNTED	NO. EGGS LAID	NO.	DAYS COUNTED	NO. EGGS LAID
1	34	1056	10	33	974
2*	6	144	11*	10	221
3	14	0	12	46	826
4	13	372	13	84	1807
5*	7	170	14	48	1429
6*	4	116	15*	15	351
7*	15	462	16*	28	782
8	88	2184	50	26	552
9	71	1613	51	33	756
56*	30	828	52	52	601
57*	16	371	53	44	652
58	52	1246	54	48	901
59*	14	304	55*	9	200
60*	59	1265	Total	476	10052
61	48	1360			
Total	471	11491	Average per day 21.12		

Average per day 24.4

* Escaped

TABLE 22

Showing the total output of eggs of the Woods Hole stock and their hybrid children when crossed to the truncate stock

WH ♀ × WH ♂			A ♀ × A ♂			B ♀ × B ♂		
NO.	LIFE OF ♀	NO. EGGS LAID	NO.	LIFE OF ♀	NO. EGGS LAID	NO.	LIFE OF ♀	NO. EGGS LAID
13	41	812	1	51	1406	7	35	835
14	51	443	2	14	403	8	38	1043
15	61	9	3	34	886	9	55	1511
16	61	896	4	31	589	10	29	740
17	61	369	5	52	1482	11	50	1204
18	16	346	6	52	232	12	25	490
19	54	776	Total....	234	5998	Total....	232	5823
20	57	931	Average number of eggs laid per day 25.63.			Average number of eggs laid per day 25.09.		
21	54	600						
22	61	1034						
Total..	517	6216						

Average number of eggs laid per day 12.02.

Average number of eggs laid per day, not considering number 15, 13.61.

animals seem to be better somatically than either parent. To be sure, their fertility inter se is much lower but it should be remembered that while the individuals belong to one generation the egg and sperm produced belong to the next.

The following suggestion is offered as a tentative explanation: It is probable that some factor or factors, let us say, regulate egg production in the normal wild fly.¹ Let us designate these as AB.

Let us assume that the truncates have dropped out some factor from this mechanism, then their formula would be expressed by Ab. The other stock used had been inbreeding in mass cultures in the laboratory and had probably lost some factor, let us say A. Then their formulae would be expressed by aB. When the two races are crossed, the two factors are brought together again, thus ABab, and the normal egg-laying function is restored. The explanation holds at least tentatively for other improvements to be seen in the hybrid. The number of factors concerned is not to be looked upon to be as simple as the formula would seem to indicate. Instead of two factors, as the formula shows, there may be many units concerned, but the principle is the same. The things lost out of the germ-plasm of one are made good by those present in the other and thus normal conditions are restored. If this is so, we should expect to find members of this species in nature that live well on to one hundred days and some that have high egg production and high fertility combined. They should produce well on to two thousand flies.

FERTILITY OF THE LONG-WINGED BROTHERS AND SISTERS OF THE TRUNCATES

When the long-winged flies thrown by the truncates are bred together their productivity is greater than their truncate brothers and sisters. This fact is brought out in table 23 where 120 is recorded as the average per pair. Their truncate brothers and sisters used for control produced an average of 77 offspring per pair. The flies were of the same ancestry and subject to the same environmental conditions. That the long-winged forms produce

¹ Whether the actual formation of germ cells, in this case eggs, or the regulatory mechanism that governs their actual maturation and discharge is involved is a problem more difficult of solution.

more offspring than their truncate brothers and sisters is borne out by table 24, in which the F_2 generation of the longs as thrown by the truncates are tested in pairs. In this experiment they gave an average of 92 offspring per pair, while 34 pairs of truncates used for control gave an average of 47 offspring per pair. (Table 1, F_{4s} .)

I have pointed out that the number of offspring produced by a pair of flies is not an infallible guide to the fertilizing power of their gametes. That the fertilizing power of the egg and sperm of the long-winged forms is greater than that of the truncate flies is supported by the following experiment, in which the eggs were isolated and 37 per cent hatched. Since I have never been able to obtain more than 24 per cent of fertile eggs from the truncates I conclude that this difference when taken in connection with the other evidence is significant. Attention is called to No. 31, table 14b, in which the fertility of a long-winged female is 38 per cent, or 14 per cent greater than her truncate sisters.

The data recorded in table 25 are too small upon which to base any very definite conclusion in regard to this obscure phenomenon,

TABLE 25

This table shows the result of testing in pairs the long-winged brothers and sisters of the truncates. For testing of truncate brothers and sisters to these, see table 1.—F 44.

NO.	TRUNCATE ♂	TRUNCATE ♀	TOTAL TRUNCATES	LONG ♂	LONG ♀	TOTAL LONGS	GRAND TOTAL
1	5	5	10	41	39	80	90
2	15	14	29	76	75	151	180
3	2	4	6	21	23	44	50
4	3	4	7	67	75	142	149
5	11	6	17	37	28	65	82
6*	—	—	—	—	—	—	—
7	12	17	29	53	59	112	141
8	7	7	14	56	56	112	126
9*	—	—	—	—	—	—	—
10	15	10	25	70	49	119	144
Total.....	70	67	137	421	404	825	962
Average....	—	—	—	—	—	—	120

* Sterile.

TABLE 24

Showing the result of breeding in pairs the long-winged individuals selected from the previous experiment, the results of which are shown in table 23.

ANCESTRY NO.	SERIAL NO.	TRUNCATE ♂	TRUNC ♀	TOTAL TRUNCATE	LONG ♂	LONG ♀	TOTAL LONGS	GRAND TOTAL
2	1	13	7	20	66	49	115	135
2	2	0	0	0	33	33	66	66
2	3	0	0	0	6	7	13	13
2	4*	—	—	—	—	—	—	—
2	5	2	5	7	8	7	15	22
2	6	0	0	0	68	42	110	110
3	7*	—	—	—	—	—	—	—
3	8	5	4	9	57	53	110	119
3	9	3	2	5	37	35	72	77
4	10	7	2	9	42	56	98	107
4	11*	—	—	—	—	—	—	—
4	12	6	8	14	59	77	136	150
4	13	0	1	1	27	42	69	70
4	14	0	1	1	41	38	79	80
4	15	3	1	4	24	27	51	55
4	16	13	15	28	72	49	121	149
4	17	15	10	25	66	53	119	144
4	18	1	4	5	8	5	13	18
8	19*	—	—	—	—	—	—	—
8	20	2	4	6	17	31	48	54
8	21	4	11	15	57	40	97	112
8	22	0	1	1	4	5	9	10
10	23	5	9	14	86	86	172	186
10	24	24	45	69	50	52	102	171
10	25*	—	—	—	—	—	—	—
10	26	1	4	5	30	41	71	76
Total....		104	134	238	858	828	1686	1924
Average...		—	—	—	—	—	—	92

* Sterile

TABLE 25

Showing the number of eggs isolated and the corresponding number that hatched on pairing the long-winged males and females thrown by the truncates

NO.	NO. EGGS ISOLATED	NO. FLIES HATCHED	NO. EGGS NOT INCLUDED
1	152	66	0
2	41	44	31
3	71	20	31
4	73	22	20
5	45	18	0
Total	382	140	82

but that the productivity of the long-winged individuals is greater than their truncate brothers and sisters I can testify from my experience with them in mass culture, and it would seem as though this increased productivity is due to the increased fertilizing power of the egg and sperm. I shall return to this question in Part IV.

BEHAVIOR OF THE TRUNCATE WING IN HEREDITY

Attention has already been called to the fact that many generations of inbreeding the truncate brothers and sisters has failed to purify this stock. The truncate always throw long-winged males and females. The long-winged flies in turn throw some truncates. Table 1 gives the number of truncates and longs that appeared in the different generations by continually selecting the truncates. From a total of 6356 there are 5469 flies with truncate wings and 887 flies with long wings; a ratio of 1 long to 6.2 truncate. During the period under investigation this ratio remained fairly constant.

When the long-winged flies thrown by the truncates were bred together there appeared in a total of 962 flies, 825 with long wings and 137 with truncate wings; a ratio of 6.2 longs to 1 truncate. When the F_2 generation of long-winged flies were bred together the different classes appear as shown in table 24. In a total of 1924 there are 1686 long-winged flies and 238 truncate flies. The males and females are about in equal proportions. This gives a ratio of 1 truncate to 7.1 long. This ratio is practically the opposite of the condition found in the truncate wing which threw 1 long to 6.2 truncates.

When the truncates are paired with the long winged wild stocks such as the inbred or Woods Hole, long wings are dominant to short, but not completely so; for while no truncate wings appear a new class of wing arises. This is a long wing with small crescent shaped piece cut out of the inner margin at the tip of each wing. These will be referred to as 'crescents.'

In the cross as given in table 3 there appeared among a total of 1784 hybrids, 41 crescent males and 35 crescent females, a ratio of 1 crescent to 21.2 longs. In the reciprocal cross from a total of 4711 offspring there were 30 crescent males and 84 crescent

females; a ratio of 1 crescent to 40.3 longs. I shall neglect this ratio in further discussion as it appears to be aberrant.

In the cross as given in table 6 a, there were 34 crescent males and 19 crescent females, a ratio of 53 crescent to 1127 long or 1 to 21.3. In the reciprocal cross, table 6b, there were 30 crescent males and 49 crescent females in a total of 1714. This gives a ratio of 79 crescent to 1635 long or 1 to 20.7.

The different classes appeared in F_2 generation of the crosses as follows. From a total of 16096 as given in the cross in table 9 there are 516 truncate males, 778 truncate females, 431 crescent males, 633 crescent females, 6881 long males and 6857 long females. In the reciprocal cross there are 273 truncate males, 332 truncate females, 315 crescent males and 436 crescent females, 4986 long males and 5458 long females. Out of a total of 27896 there are 1903 truncates and 1881 crescents. This gives a ratio of 1 truncate to 13.2 longs and 1 crescent to 13.4 longs. The ratio is practically the same in both the cross and the reciprocal. To summarize in a general way, we may say that during the period of investigation the truncates threw one long in $7 \pm$ truncates. Their long-winged brothers and sisters threw 1 truncate in $7 \pm$ longs. The new type of wing, the crescent, appeared in the crosses between the truncates and the wild stocks and in the ratio of 1 crescent to 21 longs in both the cross and its reciprocal. In the F_2 generation crescents and truncates appear in equal numbers from both the cross and the reciprocal and in the ratio of 1 to 14.

These ratios apparently do not fall under any Mendelian explanation and yet I believe that any explanation of these ratios must take into consideration the great viability of the truncate stock and my opinion is that when more facts are available this case may be found to fall under a Mendelian formula because there is evidence of segregation and because definite ratios appear according to the combinations that are made.

SEX RATIO

It is not the purpose here to enter into a discussion of sex-determination. I merely wish to bring together the data from the foregoing experiments that bear on the question of sex-ratios. Since the viability has been so great in some of my strains it seems remarkable that disturbances in the sex-ratio have not been encountered. The tables show the ratios to be remarkably constant, with a slight excess of females in almost all cases. The females emerge first and if a count is made at this time there is in almost all cases a large excess of females. At times the ratio may be as high as two or three females to one male. Toward the middle of the count, however, the ratio approaches equality and toward the end of the count the males are usually in excess and this tends to equalize the sex ratio. It is not that the first eggs laid produce females but that the egg which is to develop into a female carries its development through on an average from twelve to twenty-four hours more rapidly than the male. This is evident from the hundreds of bottles from which my counts have been made.

Different strains of these flies vary somewhat in their rate of development. The truncates always emerged from two to four days later than the wild stocks used in control. The hybrid fly carries its development through to the hatching stage more rapidly than either of the parent strains.

I have said that the sex-ratio is remarkably constant, with the females slightly in excess. This statement applies to the inbred stock, the truncate stock and the Woods Hole stock when brothers and sisters are paired. Diagram 1 shows the ratio of males to females to be 100 to 103; 100 to 103 and 100 to 107 respectively. I think that the slight excess of females is due to the factors to which reference has already been made. When the different races are crossed into each other a rather large excess of females appears. This statement also holds for the children and grandchildren when tested together in pairs. I do not believe that the early emergence of the female from the pupae will ac-

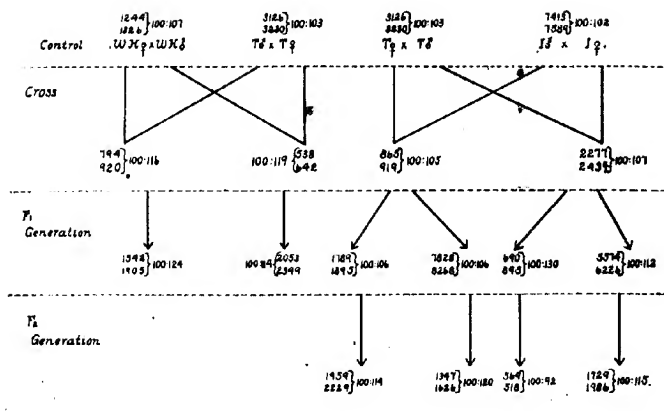


Diagram I Showing the ratio of males to females. Compiled from the foregoing experiments. T = truncate; WH = Woods Hole; I = inbred.

count for the rather large excess of females in these crosses. It should be stated here as a matter of fact that the sex ratio does not change with the age of the female. Diagram I gives the sex-ratio as compiled from the foregoing experiments.

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A QUANTITATIVE DETERMINATION OF THE ORIENT- ING REACTION OF THE BLOWFLY LARVA (CALLIPHORA ERYTHROCEPHALA MEIGEN)

BRADLEY M. PATTEN

TWENTY-FOUR FIGURES

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STATEMENT OF THE PROBLEM

In the work done thus far in animal behavior, no successful attempt has been made to apply any form of precise measurement to movements produced by light of known differences in intensity. The experiments on the blowfly larva described in this paper were devised for the purpose of making exact quan-

titative measurement of the light reactions in some suitable and easily available animal. The light was applied as two opposed beams, the intensity of which could be easily controlled and precisely measured. The responses to the stimulation produced by two beams of different intensity acting simultaneously on opposite sides of the same animal were measured, in angular deflections from an initial path of locomotion.

LITERATURE

The earliest paper on the light reactions of blowfly larvae was that of Pouchet, in 1872. He described a series of experiments with daylight and artificial light, showing the negative character of the light response of the larvae of several species of the old Linnaean family of Muscidae. A considerable portion of the paper is devoted to experiments devised to locate the light recipient organs. The fact that the light response did not disappear when the sensory cones of the anterior end were excised, led Pouchet to conclude that the imaginal discs of the adult compound eyes were the sensitive organs. This conclusion was borne out by his observations on the increase of sensitiveness with the age of the larva, which coincides with the increase in development of the imaginal discs. The following quotation sets forth his interpretation of the way in which the imaginal discs serve, not only to perceive the light but also the direction of the rays, (1872, p. 316): "La lumière, frappant, sous des angles différent, les surfaces toutes différent inclinées sur l'horizon des yeux embryonnaires, donne à l'animal le sentiment de la direction des rayons, par l'intensité relative avec laquelle ils affectent, grâce à leur incidence, les différent yeux."

This conclusion is interesting in its foreshadowing of the question of the relative effect of 'ray direction' and 'intensity difference' on orientation which grew out of the tropism controversy nearly twenty years later.

The work of Pouchet, like most studies of behavior of this period, was done on the basis of preference as shown by experimenting. Conditions were so arranged that the animals could move into regions of higher or lower intensity or of particular

color, and the results were interpreted in terms of human experience. The behavior of an animal that was assumed to have the freedom of choice was said to indicate its preference.

The students of plant behavior—because the subjects of their experiments were remote from human activities—had begun to free themselves from the anthropomorphic point of view long before the zoölogists. With their earlier analytical outlook on the natural phenomena, came an earlier seeking for mechanical explanations. In the middle of the last century, when animals were still going toward light “because they liked it,” or “because it aroused their curiosity,” the botanists were seeking to determine the nature of the mechanism involved in the reactions of plants to light.

As early as 1831 de Candolle had used the term heliotropism to describe the bending of plants toward the sun. This he believed to be due to the direct effect of local difference of intensity, growth being retarded on the more highly illuminated side. The inadequacy of this explanation was shown by Charles Darwin and his son Francis in their work on “The power of movement in plants;” these investigators demonstrated the difference between sensitive and reacting tissues and the transmission of stimuli from one to the other.

Sachs, however, did not accept the theories of the Darwins and advanced the explanation that the turning was controlled by the direction in which the rays of light penetrated the tissues of the plant. The simplicity of the theory of Sachs was very alluring, but, like many “simple mechanical explanations,” it fitted only a few of the facts.

Soon after the work of Sachs appeared, Loeb took up the study of animal reactions with the purpose of analyzing the phenomena in terms of physics and chemistry in opposition to the anthropomorphic ‘explanations’ prevalent at that time among zoölogists. His attitude is well expressed by the following quotation from one of his later works. He says ('05, p. ix) “I consider a complete knowledge and control of these agencies (which determine behavior) the biological solution of the metaphysical problem of animal instinct and will.” Whether or not future work bears out

all Loeb's conclusions is a matter of small importance beside the tremendous advance in clear thinking which has resulted from the analytical attitude he has from the first maintained.

In his earliest work bearing on the orientation of animals to light ('88), he cites the reactions of fly larvae in support of his explanation of orientation. The main interest of this work was theoretical and but few facts were added to the fairly complete account already published by Pouchet. Perhaps the most significant of these were his establishment of the restriction of the sensitive region to the anterior end of the larva and the balanced reaction to equal lights acting on opposite sides of the body. Loeb concluded that the factor of prime importance in orientation was the direction in which the rays of light penetrated the tissue, as Sachs had believed for plants. This view, however, he has since abandoned, as may be seen from the following quotation ('06, p. 130),

We started with the assumption that the heliotropic reactions are caused by a chemical effect of light; in all such reactions, time plays a rôle. We assume, furthermore, that if light strikes two sides of a symmetrical organism with unequal intensity, the velocity or the character of the chemical reactions in the photosensitive elements of both sides of the body is different; that in consequence of this difference the muscles, or contractile elements on one side of the organism are in a higher state of tension than their antagonists. The consequence is a curvature or bending of the head. This is followed by a turning of the body, kept up until the stimulus acts equally on the bilaterally located sensitive areas. When such a condition of balance is attained, the animal no longer deviates toward either side, but pursues a direct path toward or away from the source of light.

If it be true that the immediate effect of light in causing the heliotropic reactions is of a chemical nature, we should expect that it must be possible by use of chemicals to control the precision and sense of heliotropic reactions ('06, p. 131).

The striking results which Loeb ('93, '04, '06) obtained in his experiments on the chemical control of heliotropism are the strongest sort of evidence for his interpretation that light reactions depend fundamentally on a chemical reaction, the extent of which is dependent on the intensity of the light.

Loeb thus opened a new field of tremendous interest. The mechanical explanation of orientation to light became the ob-

jective point of work on light reactions. Interest no longer centered about the cataloguing of the positive or negative response of various forms, but about the developing of a general theory of light reactions.

With the possible exception of Engelmann's work on swarm spores, most of the work on behavior up to this time had dealt with mass reactions rather than with the details of individual reactions. In 1897 Jennings began a study of the aggregations so commonly found in the cultures of *Paramecium*, by observing in the minutest detail the reactions that brought a single individual into the aggregation and held it there. The work on *Paramecium* was followed by many other papers on the Protozoa and some on the lower Metazoa, all characterized by the same thoroughness in the observation of individual behavior. Such methods brought out many facts of great interest, previously overlooked.

One of the points on which Jennings lays great emphasis is that behavior does not depend on the external stimulus alone. He believes that there are various internal factors which modify the reactions to the same external conditions. Former stimuli and the reactions of the organisms to them, as well as the metabolic processes constantly going on within the animal, have their effect on the physiological state. The physiological state in turn determines to a large degree the reaction of the organism to external stimuli.

Jennings also found that in the Protozoa on which he worked there were no bilaterally located sensory areas and that the position of orientation was not one in which the median plane of structural symmetry was placed in a definite position with reference to the source of stimulation. The 'tropism theory,' as put forward by Loeb, evidently did not apply to these organisms. Jennings observed that changes in the direction of locomotion were brought about by 'motor reflexes' directed toward a structurally definite side of the organism. He characterizes this method of orientation as one of 'trial and error.' The 'varied movements' of locomotion involve contact with varying environmental conditions, selection from among these conditions is

brought about by the 'motor reflex' produced whenever a stimulus is encountered. This usually removes the animal from the source of stimulation, for the 'structurally definite side' toward which the reflex is directed is that opposite to the side on which the sensitive area is located. If the distribution of the stimulus in space is such that these reflexes hold the animal on a direct path with reference to the source of stimulation, a response resembling a 'tropism' results, but the method of its accomplishment is different from that assumed by Loeb. Jennings did not, however, believe that the orientation of all animals was brought about by the motor reflex ('06, p. 271). "In the symmetrical Metazoa we of course find many cases in which the animal turns directly toward or away from a source of stimulation without anything in the nature of preliminary trial movements."

About this time Holmes ('05) published a detailed account of the movements involved in the orientation of the individual blowfly larva. He lays great stress on the significance of the side to side swinging of the anterior end which occurs when the larva is suddenly stimulated by lateral illumination. These random movements, he believes, afford a means of selecting a favorable direction of advance. He says ('05, p. 105):

There is, so far as I can discover, no forced orientation brought about by the unequal stimulation of the two sides of the body, but an orientation is produced indirectly by following up those chance movements which bring respite from the stimulus. I do not deny that there may be an orientating tendency of the usual kind, but if there is, it plays only a subordinate rôle in directing the movements of the animal. The orientation of these forms is essentially a selection of favorable chance variations of action and following them up (p. 106). It may be said to be a form of the trial and error method minus the element of learning by experience.

Hermes has published two articles on the light reactions of the larvae and adults of the Sarcophagid¹ flies. The earlier paper ('07) is based largely on a field study of the light reactions as they affect food habits and migration. The second paper ('11) contains an account of numerous laboratory experiments in which he has brought out a number of interesting points, such as the increased rate of crawling under increasing intensity of illumi-

¹ Hermes bases his classification on Girchner's system, published in 1896.

nation, the positive reaction of aggregations of feeding larvae to a light of moderate intensity, and the circus movements performed by larvae with one side of the anterior end blackened. A valuable part of the paper is occupied with the confirmation by more exact experimental data, of several of the earlier conclusions.

Mast in his extensive treatise on "Light and the behavior of organisms" has collected practically all the important work of the earlier writers and added many experiments of his own. His original work is similar to that of Jennings in the careful observation of the details of individual behavior. One cannot fail to be impressed by the painstaking methods of his experiments, but it is difficult to see how his results invalidate the theories of Loeb so completely as he would have us believe.

Considerable space is devoted to the reactions of fly larvae. Experiments are described which bear out the conclusion of Loeb and Herms that the sensitive region is restricted to the anterior end, but none of them aid materially a precise localization of the sensitive organs. His experiments on the effect of intensity on the rate of locomotion failed to yield any very definite results, largely because they were made with horizontal lights and hence the shadow of the animal's body prevented the direct operation of the light on the sensitive anterior end. The most interesting part of his work is the series of experiments by which he establishes that, in this form as well as others on which he worked, orientation depends primarily on the intensity which operates on the sensitive surfaces, but depends on 'ray direction' only in so far as it modifies the operative intensity on the receptive areas. His analysis of orientation will be taken up in same detail in the discussion of theories of orientation.

Several authors have experimented with the effect of colored light on maggots, but the work of Gross ('13) is, by reason of the refinements of his apparatus and methods, by far the most accurate that has been done in this field. The colors used in his experiments were practically monochromatic and were accurately measured for intensity by means of a radiomicrometer. The sequence of effectiveness which he established for the larvae—green, blue, yellow, red, decreasing in the order named—is unusual in the greater effectiveness of the green than the blue.

It is interesting that in the imago the conditions are reversed, and the blue is more effective than the green.

In the work which has been done on the light reactions of the blowfly larva there is essential agreement as to the general manner in which the animal actually behaves under various experimental conditions. The interest of recent work has centered about the discussion of details of behavior which have been adduced in support of one or the other of the theories of orientation. There has been no attempt made to obtain definite measurements of any phase of the orienting reaction. The purpose of the present paper is to devise a method of quantitative measurement which shall be available in work on light reactions and to apply such a method to the orienting reaction of the blowfly larva.

APPARATUS

The apparatus used in these experiments was constructed so that the opposite sides of the animal under observation could be subjected to opposed beams of light the actual and relative intensity of which could be varied at will. The plan of the apparatus is shown in figure 1.

It consisted of a horizontal wooden frame in the form of an isosceles right triangle. As shown by the dotted lines on the diagram, this frame was so proportioned that a similar triangle measuring two meters on the base and 1.414 meters on the equal legs, could be laid out on it. Where the side bars of the frame came together to form a right angle, a horizontal platform 65 by 35 cm. was attached. On this platform, at the apex of the triangle were set up five 220-volt Nernst glowers, mounted vertically and about 5 mm. apart, each with a switch in circuit. Their position was such that no glower interfered with the light thrown on the mirrors by any other glower. When less than the five-glower intensity was desired, symmetrically placed glowers were used.

A portion of the light from these glowers passed through the horizontal rectangular apertures (3.2 by 6.1 cm.) in the screens *d* and *d'*, placed 25 cm. from the glowers.

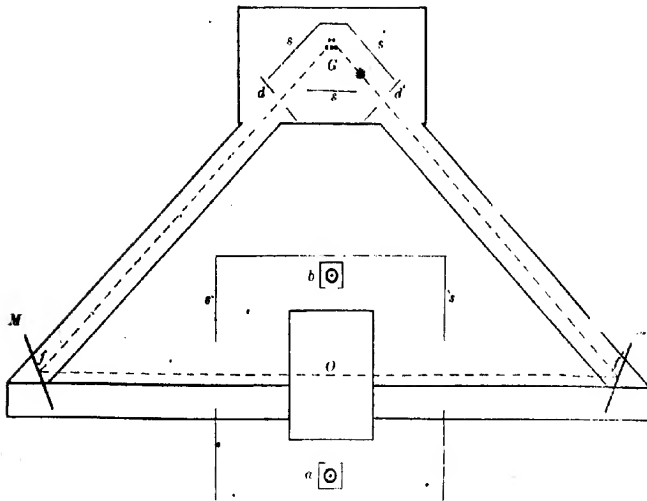


Fig. 1 Diagram of apparatus used to produce differential bilateral light stimulation. *G*, five 220-volt Nernst glowers; *M* and *M'*, mirrors; *f* and *f'* central point of mirrors; *O*, center of observation stage; dotted lines, central ray of beam of light from the glowers reflected to *O* by the mirrors; *d* and *d'*, screens with rectangular openings; *s* and *s'*, light shields; *a* and *b*, 2 c. p. orienting light with screens.

The central ray of light from the apertures fell on the centers of the vertical mirrors *M* and *M'*, which were cut from the best French glass, and, to insure uniformity, from the same piece. These mirrors were placed at such an angle that they reflected the central ray of the beams *Gf* and *Gf'* to the observation point *O* directly opposite the glowers. It will be seen that the apparatus was so constructed that the courses taken by the light over the two paths from *G* to *O* were equal in length and symmetrical in position. Furthermore, the central rays impinging at *O* were in the same straight line but came from opposite directions. The two beams of light reaching this central point should be of equal intensity, and careful photometer tests showed them to be so. By using a single source of light for both beams, the uncertainty due to the possible fluctuations in two separate sources of light was eliminated.

A horizontal stage of slate 30 by 48 cm., on which the animals were observed, was placed at the same level as the center of the glowers, and in such a position that the observation point *O* fell at its center. At either end of this stage, a 2-candle power incandescent lamp was set up at a distance of 55 cm. from the central point *O*. By means of these 'orienting lights,' (fig. 1 *a* and *b*) a negatively phototactic animal could be started from either end of the observation plate, directly across the field of light from the mirrors. Light screens around the glowers, *s*, and a larger set of screens around the observation stage, *s'*, shut out extraneous light and reduced reflection to a minimum. The orienting lights also were screened except for a small horizontal aperture throwing its central ray to *O*.

With the apparatus thus set up, a maggot could be made to move on to the observation stage, away from one of the orienting lights, so that two equally intense beams of light fell on its opposite sides. By reducing the intensity of one of the beams of light, differential bilateral stimulation could be set up, of any desired proportion or intensity. It was important to find a reliable method of reducing one of the beams of light. The episcotister, though very convenient, has been shown to be an unreliable means for the accurate reduction of light intensity (Parker and Patten '12). Several forms of gratings were tried, none of which gave a perfectly uniform field. The difficulty of securing a uniform field, with diaphragms of small enough aperture to give the desired intensity differences, was very great. The most satisfactory means seemed to be to move the observation stage to points on the basal arm of the apparatus where, according to the law $I \propto \frac{1}{d^2}$, the intensities should be of the desired ratio. Such a method, though cumbersome, gave an absolutely uniform field, and the intensity could be figured with great accuracy.²

² Hyde ('06) has computed very accurately from the law $I \propto \frac{1}{d^2}$, the amount of variation when the source of light is a cylinder of the dimensions of a Nernst glower instead of a point. For the distances used in this apparatus, the variation is from +0.03 per cent to +0.03 per cent.

The percentage differences in the two lights used in this series of experiments were $66\frac{2}{3}$, 50, $33\frac{1}{3}$, 25, $16\frac{2}{3}$, $8\frac{1}{3}$ per cent, and as a control, equality. The points on the basal arm of the apparatus at which these percentage differences exist are: $66\frac{2}{3}$ per cent, 63.9 cm.; 50 per cent, 41.4 cm.; $33\frac{1}{3}$ per cent, 24.6 cm.; 25 per cent, 17.4 cm.; $16\frac{2}{3}$ per cent, 11 cm.; $8\frac{1}{3}$ per cent, 5.4 cm. to the right or left of the central point *O*. The percentage differences are in all cases expressed in terms of the stronger light. The actual intensities of the beams are given in table 1.

With the apparatus set up for unequal opposed lights, negative animals should deflect toward the weaker light, and the deflection should be greater as the difference between the lights is increased. This deflection measured in degrees gives the desired quantitative expression of the physiological effects of the light.

MATERIAL AND METHODS

1. Cultures

The raising of blowfly larvae, and the duration of the various stages of their development under natural and artificial conditions, has been dealt with by Herms ('07, '11). He also made observations on the differences in behavior between certain of

TABLE 1

The intensities in candle meters of all the lights used in the series of experiments. The four-glower intensity is slightly lower than would be expected because the position of the glowers was such that there was a minimum of mutual heating at this intensity. The $83\frac{1}{3}$ per cent intensities do not fit in with the series because to obtain such a large difference it was necessary to re-set the mirrors.

PER- CENTAGE DIFFER- ENCE IN LIGHTS	RATIO OF LIGHTS	INTENSITY OF WEAKER LIGHT IN CA. METERS					INTENSITY OF STRONGER LIGHT IN CA. METERS				
		1	2	3	4	5	1	2	3	4	5
		glower	glowers	glowers	glowers	glowers	glower	glowers	glow- ers	glow- ers	glow- ers
Equality	1 to 1	6.32	13.9	24.6	31.5	41.1	6.32	13.9	24.6	31.5	41.1
$81\frac{1}{2}\%$	11 to 12	6.05	13.4	23.6	30.2	39.3	6.61	14.6	25.7	32.9	42.9
$16\frac{2}{3}\%$	5 to 6	5.77	12.8	22.5	28.8	37.5	6.93	15.3	26.9	34.5	45.0
25 %	3 to 4	5.50	12.1	21.4	27.4	35.7	7.34	16.2	28.5	36.5	47.6
$33\frac{1}{3}\%$	2 to 3	5.21	11.5	20.2	25.9	33.8	7.83	17.1	30.5	39.0	50.9
50 %	1 to 2	4.60	10.2	17.9	22.9	29.9	9.20	20.2	35.8	45.9	59.9
$66\frac{2}{3}\%$	1 to 3			15.4						40.4	
$83\frac{1}{3}\%$	1 to 6			7.6						46.4	
100 %	0 to ∞			0.0						24.6	

the species, a detail which has been too often neglected in work on behavior. Though the species he worked on especially, *Lucilia caesar*, and *Calliphora vomitoria*, reacted essentially in the same manner, the former was noticeably more sensitive to light than the latter. Similarly with various *Planaria*, Walter ('07) has shown well defined species differences in the light response. To avoid inconsistencies which might arise by the neglect of species differences, except for some preliminary experiments, the work was done with the larvae of a single species, *Calliphora erythrocephala* Meigen.

The larvae do not acquire their maximum sensitiveness until the end of the feeding period (Pouchet '72; Herms '11); as they approach the pupal stage they became sluggish, and if not actually less sensitive, are certainly very tedious to record. The most favorable age to make the light tests appeared to be at about the time of transition from the feeding to the migratory stage.

Like many other forms, the larvae soon become acclimated to light, and are much more sensitive if kept from one to three hours in the dark. But a long isolation from food and moisture tends to make them sluggish and to hasten the pupal stage. The best procedure is to keep the whole culture in the dark and not remove the larvae until immediately before they are to be used.

2. Variability of response

There proved to be a wide range of individual variability in the response to light, the behavior of a few of the larvae being so characteristic that they could be easily recognized by their reactions. For example, one larva had a preliminary exercise that it performed with great precision in each one of the experiments in which it was used. When placed with its anterior end away from the light, in the small wooden groove in which the larvae were started, this larva looped its anterior end directly back toward the light underneath its own body, until the 'head' emerged from under the posterior end and was struck by the full intensity of the orienting light; whereupon it promptly uncurled and crawled away from the light, responding otherwise like ordinary larvae. Out of several hundred larvae observed, I

have not seen this particular feat repeated. Though any 'extreme individuality' of this sort was uncommon, it was apparent that the rate of crawling and the response to light stimulation varied so greatly with different larvae under the same conditions that it had to be taken into account in a comparative study of a large number of individuals. To compare a larva of unknown sensitiveness, tested under one set of conditions, with an equally unknown larva tested under other conditions, would be of little value as an accurate basis for comparing the effect of the conditions. If one were attempting to find the precise effect of humidity on the time made by long distance runners, one would not try various runners of unknown speed, each under a different degree of humidity, and compare their 'times' as an index of the humidity effect. The only logical method would be to time the same runner under various conditions. His actual time would be an individual characteristic, but the relative time of the records under different conditions would be an index of the effect of humidity.

3. Standard test

But we cannot use this method on blowfly larvae, for the short sensitive period coupled with the rapid changes in the degree of sensitiveness with age, makes it impossible to complete a series of comparative experiments on a single larva. The nearest approach that can be made to such a method is to use, instead of a single larva, individuals which are as near alike as possible. For this purpose a standardization test, as it might be called, was devised and throughout the whole series of experiments only larvae testing to the uniform standard of sensitiveness were used.

■ During the experiments, each larva was kept in a separate, numbered box. The record of the standard test, as well as the subsequent trails, was made by putting a drop of very dilute methylen blue solution on the posterior end of the larva and letting it mark its own course on a sheet of paper placed on the observation stage. This method of recording the trails of larvae was used by Pouchet ('72) and Cross ('13). It has been repeatedly checked by control experiments with tap-water and appears to

change the reactions of the larvae in no way. The number of the box in which the larva was kept, in combination with the date of the experiment,³ made an identification number for all the trails of an individual larva. Thus the whole series of records was made permanent and can be referred to at any time.

The trails of the standard test were made in the following manner. A maggot was placed in front of the orienting light (fig. 1, *a*), which forced it to crawl toward the center of the stage in a line perpendicular to the ray direction of the mirror beams. When it was well on to the stage, the orienting light was turned out and at the same time the side light (fig. 1, *fO*) thrown on, thus subjecting the larva to strong illumination from the left. The lateral illumination caused the larva to turn to its own right. After this trail had been completed, the side light was turned off, and the larva at once placed at the further end of the observation plate, where it was again started across the path of the mirror beams, this time under the influence of the opposite orienting light (fig. 1, *b*). The same side light (fig. 1, *fO*) was then turned on, but as the larva was crawling in the opposite direction, the light now operated on the right photo-sensitive area, producing a turning again toward the observer's right, but toward the larva's left.

Figures 2 and 3 are photographs of actual trails made in this way, except that the methylen blue trail has been blackened with india ink to show better in the photographs. The sharpness of the bend in such a pair of trails is an index of the light sensitivity of the larva, the symmetry of the curves, an index of the photo-sensitive balance. Only those larvae orienting to the new direction of light so accurately that both trails come to lie approximately parallel to the direction of the rays, were used in making the records compiled in tables 2 to 5. The amount of variation within the limits of accurate orientation, according to this criterion, is shown in trails *a*, *b* and *c* of figure 2, all of which meet the standard requirement of sensitiveness. Though there is a clearly marked response to the new direction of light

³ The method of writing dates employed in these records is the day, month, year, notation; thus 17/6/'13 is the 17th day of June, 1913.

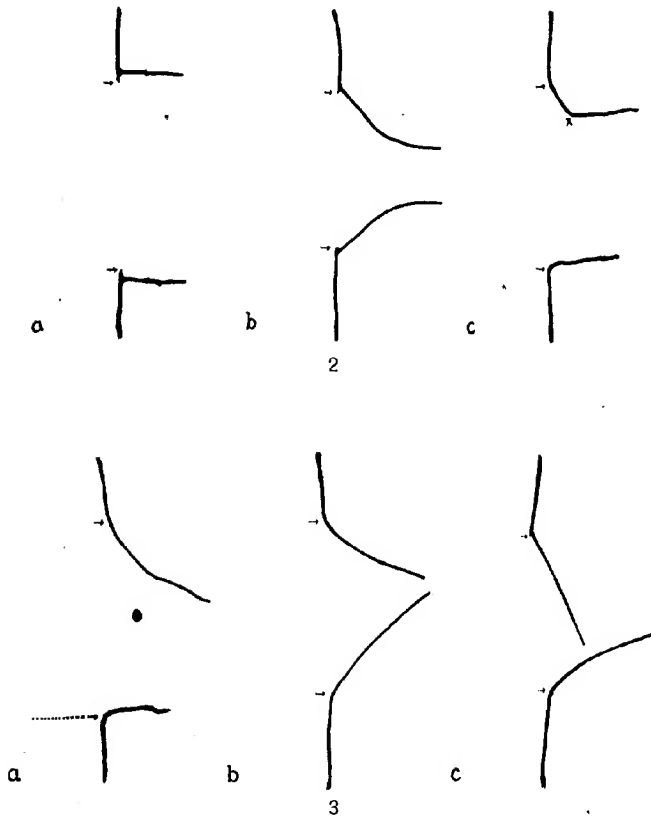


Fig. 2 Three examples of test trails which pass the standard orientation test. Each pair of trails was made by a different larva. Though there is some difference in the sharpness of orientation, each trail comes to lie parallel to the new direction of light.

Fig. 3 Three examples of test trails which do not pass the standard orientation test. Each pair of trails was made by a different larva. Though there is a response to the new direction of light in all cases, the larva fails in one or both of its trails to come to complete orientation.

in the trails shown in figure 3, *a*, *b* and *c*, these trails do not come to lie parallel to the new ray direction. These larvae were sensitive to the light but not sufficiently so to orient accurately and therefore were not used in making the records on which the tables are based.

In testing specimens from cultures at the optimum stage, from 15 to 25 per cent of the larvae were discarded because they failed to orient sharply to the change of direction in the light. Although the maggots from the same cultures were all of the same age, they were not necessarily in the same stage of development. The discarded larvae probably represented individuals that had passed, or not yet reached, their period of maximum responsiveness, rather than those which would never reach the normal degree of sensitiveness.

The larvae showing the desired accuracy of orientation were laid aside in numbered boxes and, after a rest, subjected to bilateral illumination from the mirror beams. Such a selection gave uniformity to the animals used in compiling the final results, and though it raised their standard of sensitiveness somewhat above that of the general population, it did not in any way distort the relative values of the subsequent records; on the contrary it is only by thus insuring uniformity in the material used that the comparison of results from different larvae under various experimental conditions becomes of value.

4. Plan of experiments

The plan of the experiments was to subject larvae of standard sensitiveness to lights of accurately determined intensity, with the conditions so arranged that the reactions could be measured in physical units. The rate of locomotion of the larva under the influence of light of various intensities has proved a difficult and unsatisfactory basis for a quantitative study (Mast '11, pp. 184-189). But orientation is a phase of the response to light which lends itself admirably to accurate measurement.

According to Loeb ('05, p. 2): "If two sources of light of equal intensity and distance act simultaneously upon a heliotropic

animal, the animal puts its median plane at right angles to the line connecting the two sources of light." It follows, as a corollary to this hypothesis that if the sources of light are unequal, a negatively heliotropic animal would deflect from the perpendicular toward the weaker light. This has already been demonstrated by Mast ('11, p. 179) for the blowfly larva.

By measuring in degrees the deflection toward the weaker light, physical units of measurement may be applied to the physiological phenomenon. And since with equal lights there is no deflection of the trail from the perpendicular to the line connecting the sources, whatever deflection there may be with unequal lights may be regarded as due to the effect of the difference between the lights. The starting point for a series of experiments of this sort would be at equality of the two beams of light. But it is not enough to know merely that there is no marked deflection when the lights are of equal intensity. It must be determined how many degrees from the perpendicular the aggregate response of a number of individuals falls. This necessitates not only a method of measuring each trail, but also a method of compiling the data obtained by measuring many trails made by different individuals.

The way in which we have obtained our results may be seen from a statement of (1) the method by which the trails were made, (2) the method of measuring single trails, (3) the method of tabulating the measurements.

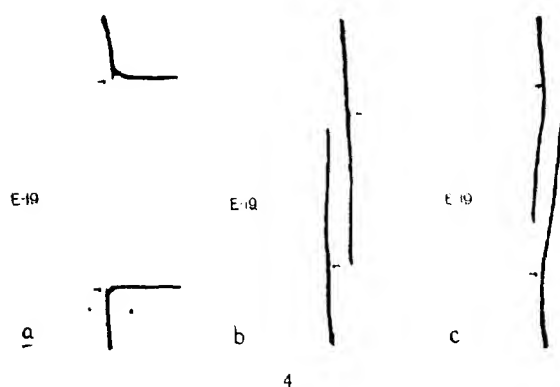
5. *Making the trails*

The handling of the larvae in making the records is of sufficient importance to be described in detail. At each end of the observation slate was a small wooden block, not shown in the diagram (fig. 1), with a groove in it leading down to the level of the slate. The blocks were located just in front of the orienting lights, the grooves extending along the line connecting the two lights (fig. 1, *a* and *b*). The larva to be tested was gently rolled from its box into the groove in front of the nearer orienting light (fig. 1, *a*), with its head away from the light. All other

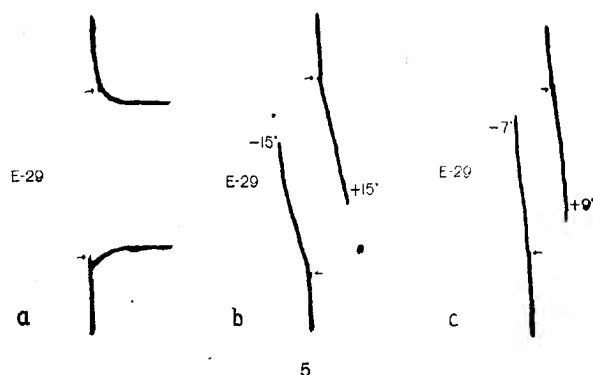
lights were shut off; the mirror beams by slides, and the opposite orienting light by a switch. The larva, started straight by the groove, was forced to move on to the paper in the direction of the orienting light, and so directly across the field of light from the mirrors. When it was well on the paper, the slides were pulled simultaneously from the two mirror beams, and at the same time the orienting light turned off. This subjected the larva to equal bilateral stimulation by the two beams of light, for the orienting light brought it into the field with its median plane at right angles to the line connecting the sources of light. Under these conditions there should be no deflection toward either light, and in fact there was none, for the larva continued in the direction in which it was started by the orienting light (fig. 4, *b*). At the end of this trail the maggot was allowed to crawl into its box, held edge down to the paper, then without being touched in any other way, it was rolled into the groove in front of the opposite orienting light (fig. 1, *b*) with its anterior end toward the center of the stage, and driven back across the field of light from the mirrors. The lateral beams were then thrown on the larva again and the second trail completed in the same way as the first. After a rest, a second pair of trails (fig. 4, *c*) was run in the same way, except that the order of running was reversed and the trail from the farther orienting light run first. These four trails, together with the test sheet, form the complete record of an individual and the unit for compiling the tables.

6. Elimination of asymmetry

But the trails do not all conform so closely to the theoretical response as those of figure 4. For example, those shown in figure 5 have a marked deflection under the same conditions of equal bilateral illumination. A careful study of this set will show the necessity of running the trails in pairs from opposite directions. In the lower trail (fig. 5, *b*), the larva deflected quite markedly to the left when the side lights were turned on, exactly as if the light from the right were stronger. But when its direction of crawling was reversed, it deflected to the right as if the left hand



4



5

Fig. 4 A set of trails made under equal opposed lights by a symmetrically responding larva; *a*, the test trails; *b* and *c*, trails under the influence of balanced lights.

Fig. 5 A set of trails made under equal opposed lights by an asymmetrically responding larva; *a*, the test trails; *b* and *c*, trails under the influence of balanced light.

light were stronger. The trails are nearly perfect mirror images of each other. Although this deflection is first to the observer's left and then to his right, it is in both cases toward the larva's left, for the direction of its crawling was reversed between trails. Such a response indicates an asymmetry in the neuro-muscular mechanism of the larva. The right side must be either more sensitive, or muscularly more active than the left for it gives a greater response to the same amount of stimulation. This asymmetry is by no means as uncommon as might be expected, in fact a certain degree of asymmetry is far more common than even an approximately perfect balance of sensitiveness. Whether the asymmetry is anatomical or physiological is a question which will be taken up later. The point we wish to bring out here is the method of eliminating the effect of such asymmetry, for it might easily be a source of serious error. Many markedly asymmetrical larvae were thrown out by the preliminary test (fig. 3, a). But many of those giving a perfectly symmetrical response in the test-trails showed marked asymmetry when run in the balanced lateral beams. The reason for asymmetry appearing in the later records, when it was not shown in the test trails, I believe to be this. In the case of the test trails, the larva is always orienting to a single light, and when orientation is attained, the anterior end of the larva is in the shadow of the posterior end. If the direction of light is changed, the larva keeps turning till it again crawls with its sensitive anterior end within its own shadow. If the 'head' swings out of the shadow, it is strongly stimulated and swings back again into orientation. Even if there were not a perfect balance of sensitiveness on the two sides, the lack of balance, unless it amounted to almost total insensitiveness of one side, would not appear under these conditions, for if, as the larva throws its anterior end from side to side in crawling, it passes beyond the boundary of the shadow, the change of intensity is sufficiently abrupt to produce a response on the less sensitive as well as on the more sensitive side, thus holding the animal within a course sharply limited by its own shadow.

When on the other hand the larva is made to crawl in a field of balance light, both lateral surfaces are illuminated, no matter

which way it turns, and the only means of maintaining its course is by a balance in the sensitiveness of the photo-receptive areas. This is clearly shown by trails such as those of figure 5. The guiding effect of the shadow is sufficient to obscure the unbalanced response in the trails of the test sheet and in the part of the trail made under the influence of the single orienting light. These trails lie almost exactly in the direction of the rays. But when the equal side lights are thrown on, which with a balanced larva should produce a direct continuation of the trail made under the influence of the orienting light, the asymmetry becomes at once evident in the deflection to the left. This larva, which gave a symmetrical test, proved to have the right side more sensitive than the left. Owing to some bilaterally unbalanced factor within the organism itself, it behaved, under the influence of equal lights, as if the right-hand light were stronger. Hence the deflection of a single trail is of little value in estimating the light effect. But turn the larva around and make it crawl into the field of light from the opposite direction (fig. 5, *b*), it still swings toward its own left side, but by reversing the direction of the crawling, the expression of asymmetry has been made to fall on opposite sides of the perpendicular to the line connecting the sources of light. If we measure deflection to the right in plus degrees and deflection to the left in minus degrees and add the two, the trails of an asymmetrical larva, run in pairs in opposite directions, are reduced to the theoretical response, and to the actual response given by perfectly symmetrical larvae.

7. Measurement and tabulation

The trails are measured as shown in the diagram (fig. 6). The lines xy and $x'y'$ are drawn through the trails at the point where the larva was when the side lights were turned on, and perpendicular to the line connecting the sources of light. A protractor is laid on the trail, with its center at the point where the lateral lights were turned on, and the deflection is measured in degrees. I have called this the angular deflection of the trail.

The measurements obtained from each individual were collected in tables such as table 2. The deflection of each trail is

entered in the appropriate column, and the sum of the total minus deflection and of the total plus deflection of the four trails is entered in the net column. The sum of the plus and minus deflections of a larva divided by the number of trails made gives

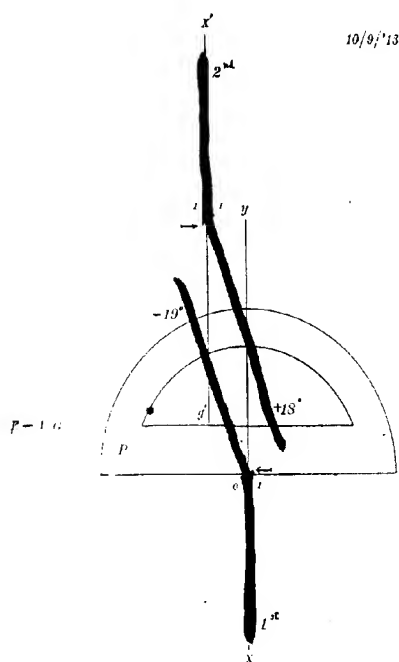


Fig. 6 Diagram to show the method of measuring trails. The lines xy and $x'y'$ are drawn through the trails at the points reached—marked by the arrows—when the side lights were turned on. The angle of deflection from this line is measured by a protractor, P . The small figures near the arrows indicate the number of wig-wag movements made when the side lights were turned on; 1st and 2nd refer to the sequence in which the trails were run; No. 7 is the box number, which, in combination with the date, gives the identification number of the larva.

the average angular deflection of the larva. The mean of the average deflections of a large number of individuals forms the final quantitative expression of the response to a given set of conditions. A study of this table shows that very few trails

TABLE 2
Equality. Four-glower intensity

NUMBERS OF THE LARVAE.	1ST SHEET		2ND SHEET		TOTAL -	TOTAL +	NET	AVERAGE ANGULAR DEFLEC- TION
	1st trail	2nd trail	3rd trail	4th trail				
No. 14, 13/5/13	+10	-16	+15	-7	23	25	+2	+0.50°
No. 18, 13/5/13	0	+6	+2	+5	0	13	+13	+3.25°
No. 20, 13/5/13	+7	-8	+18	-12	20	25	+5	+1.25°
No. 15, 13/5/13	+25	-11	-11	-3	22	28	+6	+1.50°
No. 4, 13/5/13	+6	-6	+9	-7	13	15	+2	+0.50°
No. 22, 13/5/13	0	+5	-7	-1	8	5	-3	-0.75°
No. 21, 13/5/13	-5	-3	-21	+17	29	17	-12	-3.00°
No. 16, 13/5/13	+17	-14	0	-6	20	17	-3	-0.75°
No. 11, 13/5/13	-9	-1	+13	-6	16	13	-3	-0.75°
No. 9, 13/5/13	-11	+5	-5	+3	16	8	-8	-2.00°
10 larvae					167°	166°	-1°	-0.025°
40 trails								

conform exactly to the theoretical response. Out of the forty trails, only three show no deflection whatever. But the total plus and minus of each larva comes near to cancelling, and the average of the four trails of a larva is close to zero degrees deflection. The average response of the whole set corresponds almost exactly to the theoretical response, the average deflection from the perpendicular being only -0.025° . This is astonishingly accurate when one considers that a degree on the protractor of 7.6 cm. radius used in measuring the trails was only about 1.5 mm.

The striking results of table 2 were confirmed by similar results at various intensities of the equal beams, the details of which appear later in the paper. The immediate significance of these results is two-fold. The consistent closeness of the average trail to the perpendicular, and the equal distribution of the trails on either side of it, indicate that the method of individual measurement has effectively eliminated asymmetry and placed the different individuals on a uniform basis for comparison. The close conformity of the aggregate response to the theoretical response, when the lights are of equal intensity, makes a well grounded point of departure for a whole series of experiments with opposed

TABLE 3

Measurements of the trails of figures 7 and 8 in tabular form. No. 16, 22/2/13, made the trails photographed in figure 7; No. 22, 30/1/13, those of figure 8

NUMBERS OF THE LARVAE. DATE	1ST SHEET		2ND SHEET		TOTAL —	TOTAL +	NET	AVERAGE ANGULAR DEFLEC- TION
	1st trail	2nd trail	3rd trail	4th trail				
No. 16, 22/2/13	-3	-12	-6	-11	32		-32	-8°
No. 22, 30/1/13	-29	+17	-37	+8	66	25	-41	-10.25°
2 larvae 8 trails					98°	25°	-73°	-9.125°

lights of unequal intensities. For if there is no deflection toward either side following equal bilateral stimulation, the deflection appearing under unequal bilateral stimulation may be regarded as a true expression of the physiological effect of the difference between the lights.

The same method of taking the records at equality was used with unequal lights. There is, however, one additional precaution to be observed. A given side of the larva should be subjected first to the stronger light in one pair of trails, and first to the weaker light in the second pair of trails, thus avoiding possible cumulative effects such as might result if the stronger light acted first on the more sensitive side in both pairs of trails. Asymmetry of response, though not cancelling to zero in this case, is checked out by the same method of running pairs of trails in opposite directions. The stronger light acting on the more sensitive side of the larva gives an abnormally great response; when the direction of the crawling is reversed, the same light acting on the weaker side gives a response correspondingly below the normal. The excess of one response is equivalent to the deficiency of the other, and the two average to a normal symmetrical response.

This is clearly shown by the two sets of trails photographed in figures 7 and 8, which were made under the same percentage difference in the lights, one by a symmetrical and the other by an asymmetrical larva. Tabulating these trails side by side, by the method already described, we obtain the results shown in table 3.

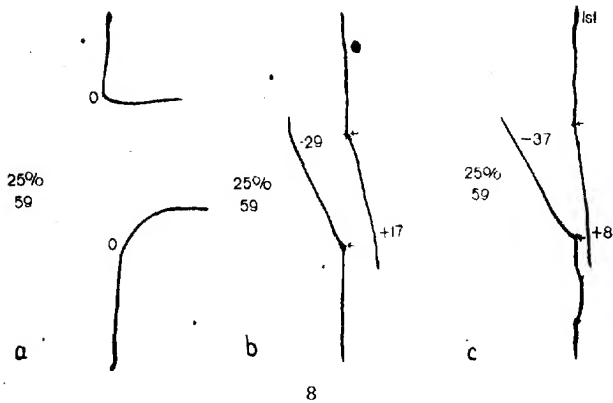
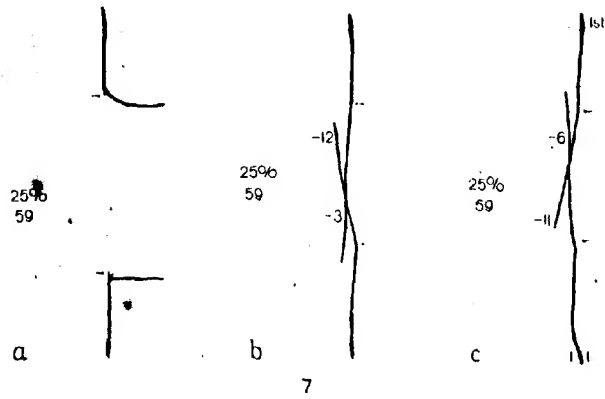


Fig. 7. A set of trails made under lights of 25 per cent difference, by a symmetrically responding larva; a, the test trails; b and c, the trails made under the influence of the opposed lights.

Fig. 8. A set of trails made under light of 25 per cent difference, by an asymmetrically responding larva; a, the test trails; b and c, trails made under the influence of the opposed light.

The total response of the four trails is nearly the same for the two larvae. Dividing the total response by the number of trails made, the average trail of the individual is obtained. This is for the symmetrical larva (No. 16, 30/2/'13), 8° , and for the asymmetrical larva (No. 22, 30/1/'13), 10.25° . A similar case at 50 per cent difference is illustrated in figures 19 and 20, page 260.

The conformity of these averages from such apparently different trails, together with the similar case already considered at equality, is convincing as to the completeness with which the disturbing effect of the asymmetry has been eliminated. I have laid special emphasis on this matter of asymmetry and the method of dealing with it, because it is important that the final results should be free from the possible cumulative effect of individual eccentricities. The average reaction of a large number of individuals, tabulated in this manner, is used to establish the angle of response under each set of conditions.

In spite of the precautions used in obtaining the records, extremely aberrant responses occasionally appeared. In all the tables, the average deviation of the individual responses from the mean of the set was computed, and when any records showed a departure of more than three times the 'average error,' they were not entered in the tables. This selection was not applied to separate trails but to the records of a larva as a whole. Either all the trails of a larva were accepted, or the larva was thrown out as an extreme variant and none of its trails were used. For these discarded trails, the trails of a fresh, tested larva, run under the same conditions, were substituted. The tables were computed both with and without this correction and the difference was found to be very slight, as the records exceeding three times the 'average error' were very uncommon, many of the tables not showing a single case.

TABULAR AND GRAPHIC STATEMENT OF RESULTS

Having perfected a system of measuring the reaction of the larvae to light, and established a fixed point of departure by means of the experiments with equal, opposed lights, we were able to attack the problem which actuated this work, namely, a tabulation of the reactions to opposing lights of different intensities, acting simultaneously on opposite sides of the animal.

It seemed advisable to begin at a well defined difference of intensity and work toward equality. Accordingly the apparatus was set up for a difference of 50 per cent between the opposing lights. By throwing glowers in or out of circuit, the absolute intensity of the common source of light could be varied at will, but however the source was varied, the relative intensity of the opposed beams remained the same. The reactions were all measured and tabulated as described in the preceding section, but as it would be impracticable to reproduce in detail over 30 tables comprising the measurements of 3000 trails it has been necessary to condense greatly the tabular statement of the results.

Table 4 was compiled from the reactions of larvae stimulated on opposite sides by lights of a constant difference of 50 per cent. The absolute intensities of the opposed beams (table 1) were varied by using from one to five 220-volt Nernst glowers as sources of light. Each column in the table shows the average response of 25 larvae, each run four times. The average angular deflection of these 100 trails is taken as the measure of the aggregate response to the special conditions. Table 4 is a condensation from five tables such as table 2, each column in table 4 representing the "Average angular deflection" column of table 2. In table 5 the statement of the measurements is still further condensed. By comparing tables 4 and 5 it will be seen that the 50 per cent column of table 5 corresponds to the "Averages for 100 trails" given at the bottom of table 4. Table 5, therefore, including as it does similar summaries of the measurements at all the other intensity differences used, is a tabular statement of the final results of the whole series of experiments.

TABLE 4
Angular deflection at 60 per cent difference

1 GLOWER	2 GLOWERS	3 GLOWERS	4 GLOWERS	5 GLOWERS
<i>Averages from four trails of a single larva</i>				
7.25°	19.25°	13.25°	20.25°	12.75°
19.00°	28.50°	19.25°	19.25°	15.50°
9.75°	21.75°	20.50°	24.25°	15.75°
25.50°	20.00°	34.00°	17.25°	29.25°
13.50°	13.50°	26.50°	19.50°	11.50°
24.25°	24.25°	22.50°	18.50°	16.75°
25.75°	32.00°	17.25°	19.00°	21.25°
26.25°	23.75°	18.50°	19.25°	19.50°
13.25°	23.25°	29.50°	19.50°	18.00°
21.75°	19.50°	16.00°	19.25°	21.00°
25.00°	13.25°	25.75°	19.75°	17.25°
27.75°	29.00°	31.50°	20.00°	24.00°
21.25°	21.25°	15.25°	17.00°	10.75°
18.75°	15.00°	22.25°	16.50°	23.00°
17.25°	17.25°	15.50°	28.50°	23.00°
23.75°	15.50°	11.50°	17.25°	9.00°
15.75°	23.00°	17.25°	8.75°	16.75°
21.75°	31.75°	27.25°	14.75°	14.50°
26.25°	27.25°	18.50°	16.00°	22.75°
23.75°	25.75°	17.00°	20.50°	21.00°
22.25°	20.00°	33.50°	17.75°	20.50°
10.00°	34.75°	18.50°	17.25°	22.75°
10.50°	24.50°	10.75°	20.25°	26.00°
9.25°	12.25°	14.00°	29.50°	24.75°
27.00°	20.00°	16.75°	27.25°	22.25°
<i>Averages for 100 trails</i>				
19.46°	22.28°	20.52°	19.88°	19.25°

TABLE 5
Based on the measurements of 3000 trails, showing the average angular deflection at five different absolute intensities and nine relative differences of intensity

	EQUAL- ITY	8½ PER CENT	16½ PER CENT	25 PER CENT	33½ PER CENT	50 PER CENT	66½ PER CENT	83½ PER CENT	100 PER CENT
1 Glower.....	-0.55°	-2.32°	-5.27°	-9.04°	-11.86°	-19.46°			
2 Glowlers.....	-0.10°	-3.05°	-6.12°	-8.55°	-11.92°	-22.28°			
3 Glowlers.....	+0.45°	-2.60°	-3.65°	-8.73°	-13.15°	-20.52°	-30.90°	-46.81°	-77.56°
4 Glowlers.....	-0.025°	-2.98°	-6.00°	-9.86°	-11.76°	-19.83°			
5 Glowlers.....	-0.225°	-2.925°	-5.125°	-8.30°	-10.92°	-19.25°			
Average.....	-0.09°	-2.77°	-5.75°	-8.86°	-11.92°	-20.28°	-30.90°	-46.81°	-77.56°

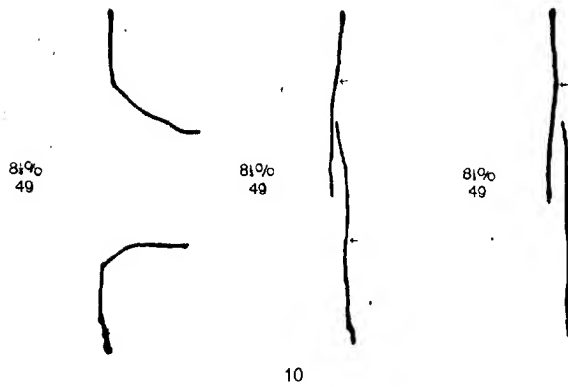
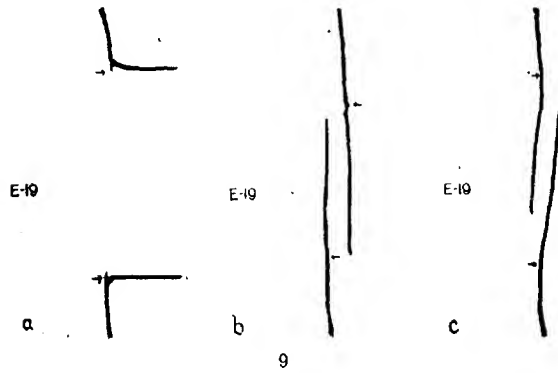


Fig. 9 The complete record of an individual larva run at equality, selected to represent the average deflection of all the trails made under the influence of equal lights.

Fig. 10 An average set at $8\frac{1}{2}$ per cent difference

Figures 9 to 14 show graphically the amount of deflection occurring at the various intensity differences. They are photographs of actual trails, selected because they represent the average deflection of all the trails run at the stated intensity difference.

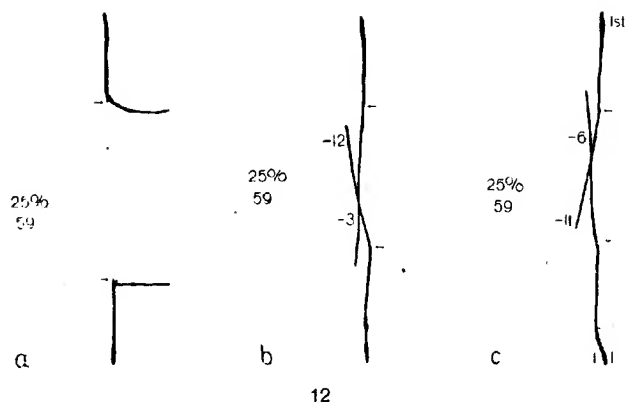
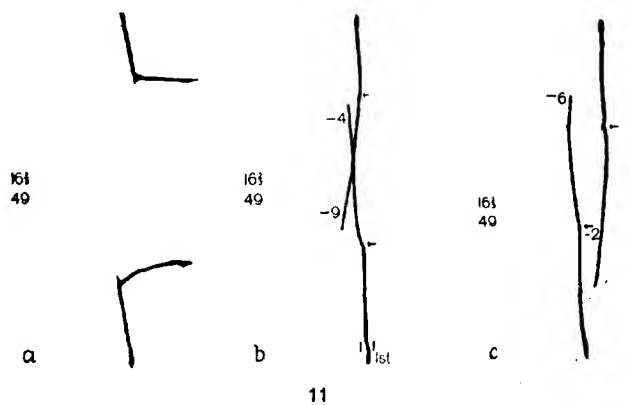


Fig. 11 An average set at 16 1/2 per cent difference

Fig. 12 An average set at 25 per cent difference

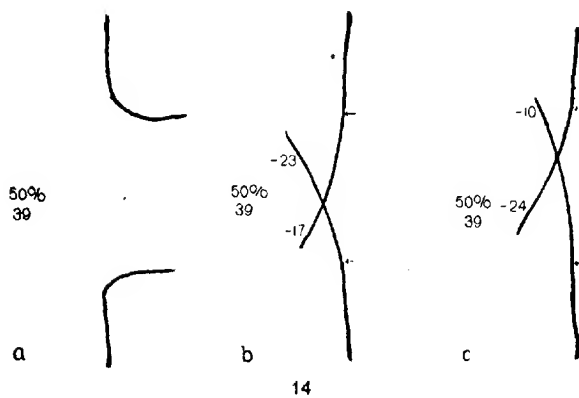
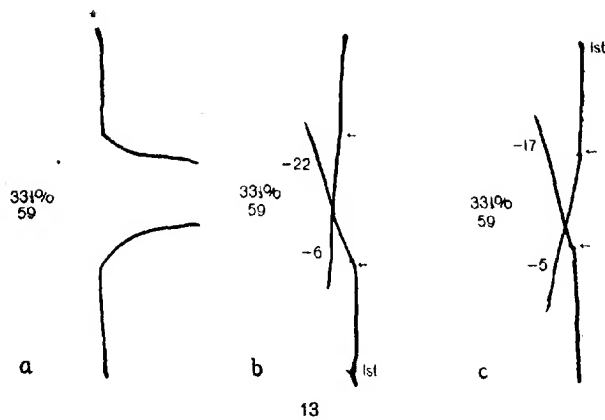


Fig. 13 An average set at $33\frac{1}{3}$ per cent difference

Fig. 14 An average set at 50 per cent difference

In the subsequent theoretical discussion, the data have been somewhat rearranged according to the point under consideration. They afford (1) the possibility of constructing a curve of reactivity to differential bilateral stimulation; and (2) a means of arriving at several facts of interest in connection with theories of orientation, because the method of light control gave an opportunity to measure the simultaneous responses of opposite sides of the same animal to equal stimuli or to stimuli of known differences of intensity.

The constancy of the angular deflection at a given intensity difference (table 5) even though the absolute intensity of the lights was varied from one to five glowers, strongly suggests that the response follows the principle of the Weber-Fechner Law. A consideration of this phase of the reactions, however, has been reserved until further experiments can be made.

DISCUSSION

1. The scale of reactivity

a. Determination. From the data already presented a scale of reactivity covering the special conditions of these experiments may be determined for the blowfly larva. It has been shown that under equal opposed lights, there is no deflection toward either side in the aggregate response, and that if the intensities on opposite sides of the animal are made unequal, a deflection appears which becomes progressively greater as the inequality of the lights is increased. Apparently the relative difference of intensity determines the amount of the deflection, for it will be seen by referring to table 5 that the deflection remains practically constant for the five different absolute intensity determinations made at each of the percentage differences from equality to 50 per cent. The average of the five absolute intensity determinations at a fixed relative difference of intensity would therefore represent very accurately the aggregate response at the given ratio and the value of the average as determined for each percentage difference between the lights may be used to locate points on a curve which may be regarded as the blowfly

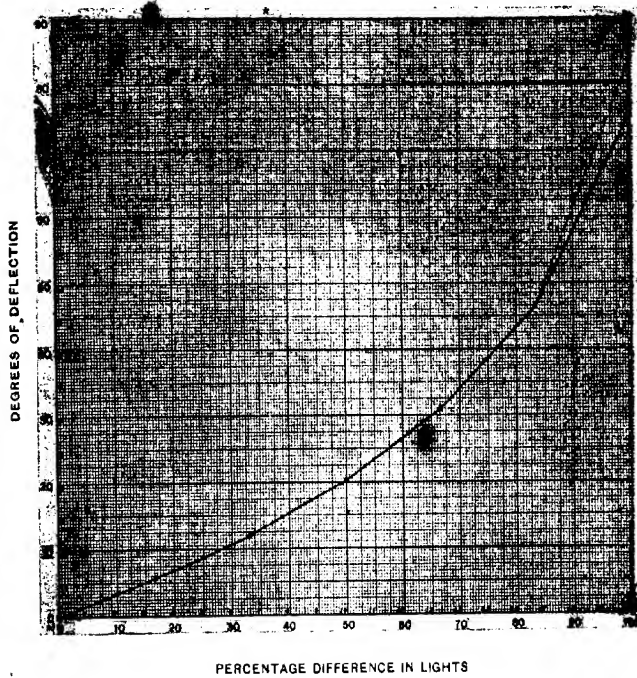


Fig. 15 A curve representing the angular deflection under opposed lights of graded differences of intensity, based on the measurement of 2600 trails; see also table 5.

larva's scale of reactivity to differential bilateral stimulation by light. Such a curve has been constructed in figure 15, plotting angular deflection along the axis of ordinates and percentage differences along the axis of abscissas. Figure 16 shows nine pairs of trails made at the nine intensity differences used to plot the curve of figure 15. These particular trails were selected because they coincided with the average deflection of all the trails run at the stated differences of intensity and therefore give a very clear picture of the experimental results on which the curve was based.

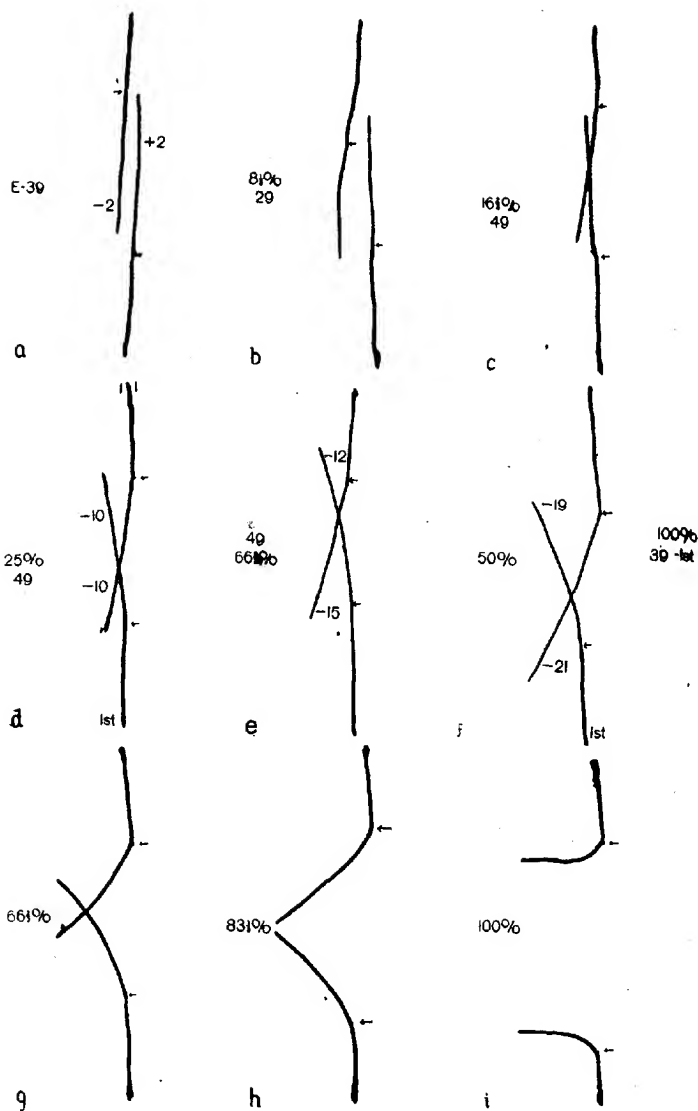


Fig. 16 A series of trails showing the progressive increase in angular deflection with increasing intensity differences between the opposing beams of light. Each pair of trails coincides with the average deflection of all the trails obtained at the stated differences of intensity; a, equality; b, $8\frac{1}{2}$ per cent difference; c, $16\frac{1}{2}$ per cent difference; d, 25 per cent difference; e, $33\frac{1}{3}$ per cent difference; f, 50 per cent difference; g, $66\frac{2}{3}$ per cent difference; h, $83\frac{1}{3}$ per cent difference; i, 100 per cent difference.

The consistency with which the deflection remained constant at various intensities of a fixed ratio made it seem unnecessary to run the whole series of intensities to establish the last three points on the curve, so the three-glower intensity alone was used at $66\frac{2}{3}$, $83\frac{1}{3}$ and 100 per cent difference. A difference of 100 per cent meant that one of the side beams should act against darkness, which was exactly the condition under which the preliminary trials were made. Accordingly, to establish this point, 200 of the test trails were measured. The deflection under these conditions should, theoretically, be 90° . But this is not the case experimentally, largely because of the time factor involved in orientation. The new orientation, even though it may be eventually a perfect alignment with the rays of light, is seldom instantaneously accomplished; and as locomotion is continued while the larva is orienting, the angle, if measured from the point where the light is turned on, is less than 90° . Figure 2 shows the varying angles in which the trails eventually come to orientation. But the time factor is of importance only when the relative difference is very great, and the amount of turning to be accomplished before orientation is attained is large. To ascertain its effect in the 100 per cent set, trails were measured in two ways: first, exactly as all the other trails were measured (fig. 6, p. 234); and again by measuring the final angle of orientation. Take, for example, the trails of figure 2, a, b, c; according to the latter method of measurement, all of them eventually attain a 90-degree angle to the line of starting; but according to the former method, the time taken in orienting lessens the angle measured. The deflection at 100 per cent, measured as the smaller angles were measured was 77.57 degrees; the deflection of the same trails measured by their final orientation was 83.78° .⁴ The difference of 6.19° between the sets represents the time factor in a change of orientation through 90° . The results of the first

⁴ The measurements of the "final angle of orientation" were made at the point where the trails crossed the scale of the protractor of 7.6 cm. radius used in the other measurements. Had the measurements been made after the larva had been allowed to crawl through a longer distance, the determination for this set would have been nearer 90° .

method of measurement are shown in the curve, as a solid line, and those of the second as a dotted line (fig. 15).

b. Application. With a series of measurements of this sort established, not only the actual response at certain fixed intensity differences is known, but the consistent progression of the values of these determinations makes it possible to predict by reference to the curve plotted from them the response that would be evoked under any intermediate degree of stimulation. This should be of practical value as a comparative physiological measure of other experimental results.

For example, the responses of the blowfly larva to colored lights have been carefully worked out by Gross ('13, p. 475): "The colored lights . . . were obtained by cutting down the spectrum by means of diaphragms of blackened cardboard with narrow vertical slits of appropriate size." The lights used were therefore practically monochromatic, while the intensity of the beams was accurately measured by the radio-micrometer.⁵ Thus the two greatest errors commonly present in colored light work (i. e., the use of mixed light due to imperfect color screens, and the inaccurate determination of the intensity) were eliminated at the start.

Gross subjected the larvae to opposed beams of light of different wave lengths but of equal radiant energy, and recorded the deflection made toward one or the other of the lights. His figure summarizing the reactions under opposed green and blue lights of equal intensity is reproduced in figure 17. I have added to his figure the labelling of the sectors in degrees, and the pair of heavy lines which represent the average deflection.* In making his records, larvae were started in the green light and allowed to crawl in the direction of its rays, along the 90-degree line of figure 17, until they reached the center of the circle. Here the blue light was turned on from the opposite side. The course then taken by the larva under the two opposed lights was recorded by indicating the sector through which it emerged from the circle. Figure 17 records in this way the points of emer-

* For description of method by which the colored lights were equalized by the radiomicrometer, see Day, 1911.

gence of 46 larvae. Thirty-four emerged on the semi-circle toward the blue, and twelve on that toward the green. The trails above the horizontal (90°) diameter were made by turnings to the left, those below by turnings toward the right. If these trails had been measured in degrees of deflection from the vertical diameter,

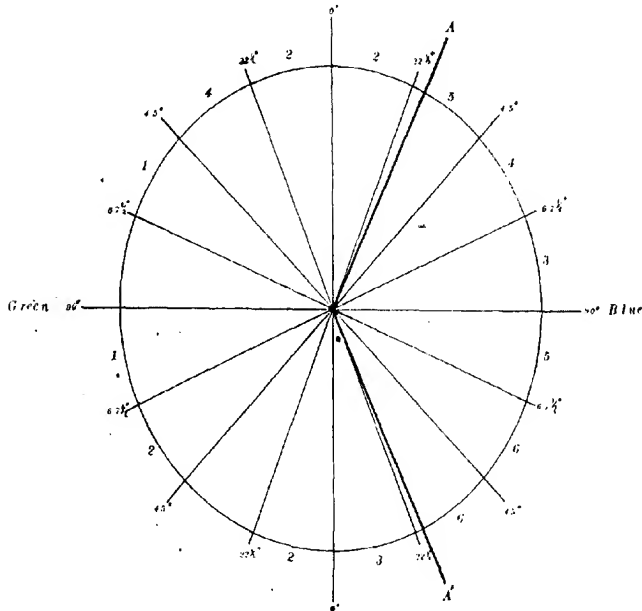


Fig. 17 A diagram to show the deflection of the blowfly larvae when subjected to opposed green and blue lights of equal intensities; after Gross, slightly modified. The numbers around the circle indicate the number of larvae that emerged through a sector. The heavy radii *A* and *A'* indicate the average angular deflection as approximately computed from the data presented by Gross.

the results would have been directly comparable to those presented in this paper for white lights. This deflection may, however, be approximately computed from the data of figure 17, by giving each trail the mean angular value of the sector in which it emerged from the circle. The trails to the left of the vertical diameter would be measured as minus, and those to the right as

plus. The sum of the plus and minus deflections, divided by the total number of trails, would give the average angular deflection toward the less effective color. The fact that certain of the larvae turned toward the color which in general acted more strongly, I believe to be due to the same asymmetry of responsiveness which, in my own experiments, caused the deflections toward the stronger of two white lights. The lack of balance of the larva in such a case would more than offset the difference in the colors, and the less effective color acting on the more sensitive side would produce a reflection toward the more effective color. In a large number of trails, however, this unstable asymmetry in sensitivity would tend to balance out, and the average deflection would be a reasonably accurate expression of the relative stimulating powers of the two colors. In the case of green opposed to blue, the average angular deflection was about 24° away from the green toward the blue. This angle of orientation could be attained by a turning either to the right or left and is represented by the heavy lines *A* and *A'* in the figure. As Gross has pointed out, this deflection is due to the qualitative difference between green and blue lights, for by actual physical measurement the intensities of the lights are the same.⁶ Comparing the angle of deflection under green and blue lights with that under unequal white lights, the angle of response corresponds to that produced when the difference in the intensity of the white lights is 56 per cent (fig. 15). In other words, green light is about twice as effective as a stimulus for the blowfly larva, as blue light. The values in the other color combinations could be estimated in the same way, but without access to the original records such computations would be only approximate and have therefore been omitted. There appears to be no other way in which the relative effectiveness of different pairs of colors can be estimated quantitatively. The method is presented more in the hope that it may prove of value in future work, than for the significance of the approximate relationship pointed out in this special case.

⁶ Gross also showed that with opposed lights of the same color and intensity, the average trail lay perpendicular to the line connecting the sources of light, thus establishing his zero point for the deflections obtained under different colors.

2. Theories of orientation

a. Relation of phototaxis and photokinesis. Among organisms that are visibly affected by light, there seem to be two distinct types of response. In some forms light appears to act as a stimulus to activity without any directive effect on position or locomotion. In other forms, light produces a definite orientation usually accompanied, in motile organisms, by locomotion toward or away from the source of light. For the first type of reaction, I have used the term 'photokinesis' (Engelmann '83); for the second, or directive response, 'phototaxis' (Davenport '97).

Holt and Lee ('01) maintained that the motor reactions of organisms to stimulation by light were not of two kinds, namely reactions to the intensity of light and reactions to the directions of its rays, but that light acted as a stimulus through its intensity alone, the direction of its rays serving merely to modify the intensity. With this conclusion I am in entire agreement. The terms 'phototaxis' and 'photokinesis,' as used in the following discussion, are applied *not* to reactions to ray direction and intensity respectively but to different types of reactions to the intensity of light.

It appears from the work of Bancroft ('13) on *Euglena*, that light may operate as a stimulus either through its constant intensity, or through changes of intensity. In *Euglena* at least the mechanisms responding to constant intensity and to changes of intensity are distinct and separately modifiable. It should be clearly borne in mind throughout the following discussion that the term 'photokinesis' is not used synonymously with 'Unterschiedsempfindlichkeit' but to include any activity induced by light, *which does not result in orientation*, whether the activity be produced by constant intensity or by changes of intensity.

Probably the fundamental response to light is of the kinetic sort, that is, activity induced by light without the operation from within the organism of factors which screen or intensify its direct action, or of factors which indirectly distribute its effects. In such cases movement persists until fatigue ensues, or if there is a region of the environment where the stimulating agent is

of an intensity which does not produce a reaction, it persists until fortuitous movement takes the stimulated organisms thither with resulting aggregation in the so-called "region of optimum intensity."

It may be assumed that in this phenomenon the animal is so attuned to light that at some degree of illumination internal and external conditions are in equilibrium and 'stimulation' ceases. The intensity at which a particular form is unstimulated apparently depends on some internal factor bound up with the ultimate make-up of the organism. It may not be any more uniform than the amount of potassium or any other chemical present in the protoplasm of various animals.

In many organisms, the kinetic response to light is the only one present. The directed reaction, however, is never present without the kinetic response. It is not a primary phenomenon; it is the kinetic response with something added to it that comes from the association of the response with a specific organic structure, a structure that may localize the access of light and the material most sensitive to it, or that may localize the reaction. As a result, movements are restricted to locomotion in a more or less definite direction. It is not, therefore, in the fundamental reaction to light that the difference between photokinesis and phototaxis lies, but in the presence in the latter of factors which localize the action of the light or which limit the direction of the response.

b. Orientation in the blowfly larva. The orientation of the blowfly larva was taken up in the hope that a careful analysis of its typical directed response would yield some evidence as to the factors involved in phototaxis. Mast ('11) has devoted considerable space to the consideration of orientation in this form. His experiments are carefully carried out and accurately recorded. I have made free use of his results in this discussion.

The process of orientation under a single horizontal beam of light is the simplest aspect of the problem. But before this can be approached, the details of normal locomotion must be considered (Mast '11, p. 176).

The blowfly larvae move from place to place entirely by means of muscular contractions. They proceed somewhat as follows: the anterior end is raised, thrust forward toward one side, fastened to the substratum, and then the posterior end is pulled forward, after which the anterior end is again raised and thrust forward, now toward the opposite side, fastened, and the posterior end again drawn up. (I may add here that the posterior end is not merely pulled forward, but is also shoved ahead by the setting of the spines of the creeping pads.) The anterior end is thus turned alternately toward the right and left quite regularly during the process of locomotion. The extent of this lateral movement varies much, but it is usually great enough so that the extremity of the anterior end is nearly at right angles to the direction of locomotion.

If the larva is subjected to lateral illumination, as it crawls along in this manner, it may orient in one or two ways: it may stop crawling, raise the anterior end more than in the usual advancing motion, and wave it from side to side, finally fixing it away from the source of light and rapidly swinging the body into alignment; or it may proceed without any interruption of locomotion and without the conspicuous waving of the anterior end, simply swing a little more widely away from the light than towards it, as it extends the head first to one side and then to the other in the normal crawling movements. The most characteristic feature of the first method of orientation is the swaying of the raised anterior end from side to side. The term 'wig-wagging,' which Walter ('07) applied to a similar process in *Planaria* may be used to characterize this form of reaction. The swinging may be first toward the light and then away, or vice versa; it may be a single movement, followed immediately by locomotion in the direction in which the anterior end is fixed, or it may be repeated several times before locomotion is resumed. Whatever the details, and there are very many different individual modifications of the behavior, the result is a sharp turning of the anterior end away from the light, followed by the orientation of the rest of the body.

The function of the wigwag movement in orienting the larva is not altogether clear. Holmes believed that the direction in which the swinging first occurred bore no relation to the direction from which the stimulus came, but that the swinging was rather

one of a series of 'random movements' induced by the stimulus and served to 'try' the conditions on the different sides of the animal. He says ('05, p. 105): "If a strong light is thrown upon a larva from one side it may swing the head either toward or away from the light." Mast, repeating the experiment, recorded the direction in which the anterior end first turned, in a large number of trials. His table ('11, p. 189) shows a total of 177 turns away from the light and 165 towards it. At first sight this would lead one to conclude that the direction of turning is little influenced by the direction from which the light strikes the animal. But other experiments at different intensities did not confirm these observations, which like those of Holmes were made under intense lateral illumination. Mast found that when a larva was subjected to a moderate intensity of illumination, the turns away from the light were much in excess. This, he interprets in the following way ('11, p. 191):

When the larvae are first exposed to sudden lateral illumination in direct sunlight, they respond immediately by throwing the anterior end toward one side violently, no matter in what position this end chances to be. If it happens to be directed from the source of light when the sunlight is flashed upon the organism, it turns toward the source of light, and if the sunlight is immediately intercepted after the larva turns, it will continue in the direction toward which the anterior end points; if it is not intercepted, the anterior end is thrown in the opposite direction, and then the larva may follow this turn and become oriented immediately, or it may swing the end back and forth a few times before becoming oriented. If the anterior end faces the light when it is exposed to the sun, it is first thrown in the opposite direction and orientation takes place just as described above. The anterior end is thus turned in the direction opposite to that in which it is when the exposure is made. It is therefore evident that under these conditions the larvae will turn toward a strong unilateral illumination as often as from it.

Apparently this is not the case when the light is less intense (Mast '11, pp. 191, 192):

If the larvae are carefully observed when they are suddenly exposed to lateral illumination by diffuse light, it is found that they respond immediately only if the anterior end is turned toward the source of light when the exposure is made. If this end is in any other position, there is no reaction whatever until the organism, in its normal process of

locomotion, extends it toward the source of light. Then it is at once turned from the light to such an extent that it frequently makes a right angle with the posterior end. Later it is swung back but only part way. . . . But why does the organism turn toward the light, if the lateral illumination is very intense? Whenever the larva is stimulated, it turns the anterior end in a direction opposite to that in which this end is when it receives the stimulus. The tip of anterior end is relatively very sensitive; in diffuse light the larvae are stimulated only when this end is extended and fully exposed, but in very intense light, owing to the translucency of the surrounding tissue, it is stimulated no matter in what position the anterior end is; consequently if this end is turned from the source of light when the organism is exposed it is at once turned sharply in the opposite direction, i.e., toward the light.

This explanation of Mast's not only clears up an apparent inconsistency in experimental results, but also establishes a point the significance of which might be further emphasized. When the anterior end of the blowfly larva is stimulated by being turned toward the light, a contraction takes place on a *physiologically definite side*—the side on which the muscles are passively stretched.⁷ This is very suggestive of the motor reflex of a form like *Stentor*, where stimulation of the light sensitive region produces a "jerking toward a *structurally definite side*," the aboral pole. In one case the organism in its locomotion is put in touch with various regions of its environment by the swinging from side to side of a bilaterally sensitive anterior region, in the other case by a spiral turning which brings the single sensitive region first to one side and then to the other. In the blowfly larva, when the 'head' is swung to the left, it is partially shaded from a light coming from the right; when a *Stentor* rotates so its oral pole lies toward the left it also is partially shaded from the light coming from the right. The change of effective intensity when the sensitive region is turned from the shaded position toward the light produces a motor reflex which involves a change in the direction of locomotion. The reflex is repeated until a direction of locomotion is attained in which the changes

⁷ The tendency of a passively stretched muscle group to contract more readily than muscles already partially contracted is familiar in many reflexes, not only in cases where a nerve net is involved, but often where there are definite afferent and efferent nerves (see Uexküll, '09, chapter, *Die Seeigel*; Howell, '12, p. 21, etc.).

of intensity no longer occur. In both organisms, such a condition is brought about by an alignment of the axis of the body with the direction of the rays. In *Stentor*, when the alignment takes place, the spiral turning no longer produces changes in the amount of stimulus; when it is attained in the blowfly larva, the shadow of the posterior end of the body produces a shaded field in which the swingings of the anterior end do not involve any considerable changes in stimulation unless they are carried beyond the shadow, when the resulting motor reflex throws the head back into the shadow again.

Instead of orienting by the method just described, which is perhaps the most characteristic, the larva may swing into alignment with the rays of light by a different method. If a larva is suddenly subjected to a light from the side there is, in this second method of orientation, no cessation of locomotion and no wigwagging. The anterior end simply swings a little more to the side away from the light than it swings in the normal locomotor movements, and the orientation is brought about gradually instead of immediately. Under the same conditions, one larva may orient by one method and the next larva by the other method. Moreover the same larva may use both methods of orientation. In figure 2, c, the upper trail was made by a combination of the two methods. When the light was turned on from the left, a gradual bending of the trail appeared, due to wider turnings away from the light than toward it. This was kept up to the point at which the trail turned suddenly (fig. 2, c, *). Here the larva stopped crawling, raised its anterior end, and performed several wigwag movements; finally it fixed its anterior end directly away from the light, and immediately came to a perfect orientation. What the cause of the sudden change of tactics was, I cannot say, but the sharpness of orientation at the place where the wigwag movements were made, in contrast to the gradual turning up to that point, is very apparent. The difference between these two methods of orientation, however, is not fundamental. The greater extent of the swingings termed 'wigwag movements' naturally brings about orientation more abruptly. In the case where the swinging is not so pronounced,

either because a lesser stimulus is acting or because a particular larva is less sensitive, it may become linked with the side-to-side movements of normal locomotion. Here the reflex contraction takes place just as before, but instead of being a separate, conspicuous wig-wag motion, it appears as a contraction added to that involved in the side-to-side swinging of the head in normal locomotion. This added contraction appears, of course, on the side away from the light and its effect is to produce a gradual turning away from the light until orientation is attained. In both cases when the anterior end is stimulated by a change of intensity, it swings in the opposite direction to that in which it was when the stimulus was received.

These two methods by which the blowfly larva orients to a single light are of such a nature that certain authors have called the process, orientation by 'trial and error.' Undoubtedly if we free the term from its original association with primitive intelligence, as they have attempted to do, it characterizes certain features of the orientation. But there are phenomena involved in the orientation of the blowfly larva to lights from two sources for which this interpretation is not adequate. The movements of a bilaterally illuminated head involve a distribution of the stimulus on the sensitive surfaces entirely different from that produced by the movements of the head when there is but a single source of light. A movement of the anterior end toward a single light produces a sudden increase in light intensity on the sensitive surfaces which is followed by a 'motor reflex,' throwing the head back into the shade of the body again. Motion toward the light is automatically prevented by the intervention of this reflex.

But with two lights of equal intensity acting on the larva from opposite sides, a swaying of the anterior end will encounter no changes of light intensity.³ Under these conditions there is,

³ The sources of light in these experiments were at such a distance from the observation stage that the change of 2 or 3 mm. from the central point, which the trial movements involved, would produce less than 0.3 per cent difference in the opposing lights. The minimum difference at which a definite change in response was detected was about 5 per cent.

therefore, no way in which the animal can 'select' from among the different intensities produced by its change of position, as the 'trial and error' interpretation demands.

Mast believes that the shading of the sensitive region by contraction, alternating with its exposure by extension provides changes of intensity which are effective in bringing about orientation. This explanation does not apply under conditions of bilateral illumination, for the change of effective intensity would be the same in whatever direction the anterior end was extended.

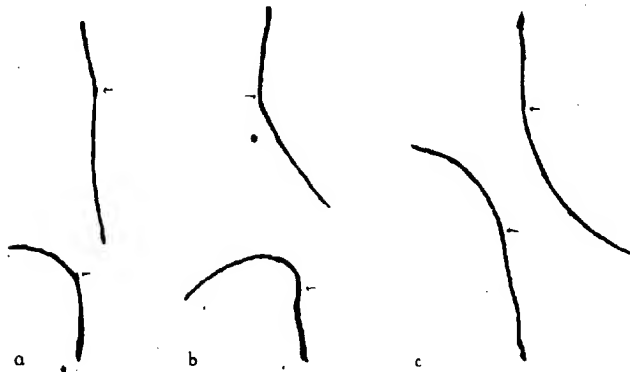


Fig. 18 Trails made by an asymmetrically sensitive larva under non-directive light; a, the test trails to horizontal lights, showing the asymmetry of response; b and c, trails of the same larva started in the orienting lights and subjected to vertical illuminating at the points marked by the arrows.

A definite orientation by the method of 'trial and error' where the 'trials' all result in the same amount of stimulation is hardly possible. Apparently the only explanation available is some form of the hypothesis advanced by Loeb, that equal intensity operating on symmetrically placed photosensitive areas produce a symmetrically distributed response. There is considerable experimental evidence that may be advanced in support of this view. Herms ('11, p. 207) blackened one side of the sensitive anterior region and found that when the animals so treated were subjected to non-directive light from above, typical circus movements were

produced with the blackened side toward the center of the circle. I tried a similar experiment with larvae that showed a markedly asymmetrical response to equal horizontal beams of light by subjecting them to light from above. The tendency to turn toward the less sensitive side was very marked. The turning, under non-directive light, of larvae that have been made artificially asymmetrical in their sensitiveness to light, and the similar turning of larvae naturally asymmetrical in their sensitiveness is hardly to be explained on any other basis than the assumption that unequal stimulation of opposite photosensitive areas is proportionately transmitted to the muscles concerned in locomotion. A significant fact in this connection is that the balance of reactivity in many of the larvae was found to fluctuate; a larva, which in one experiment gave a symmetrical response, often giving soon afterwards under the same experimental conditions, an asymmetrical one. Figure 20 (p. 260) is a photograph of the test trails, and six pairs of trails made by the same larvae under a constant intensity difference. The response is first 'left-handed,' *b*, (i.e., stronger to the left than to the right, implying that the right photosensitive area is more sensitive), then right handed, *c*, then symmetrical, *d* and *e*, then right-handed again, *f*, and finally symmetrical, *g*.

Walter ('07, p. 59) suggested that the asymmetry of response to light that he observed in planarians was due to internal irregularities. Undoubtedly asymmetry of response depends in many cases on anatomical differences in the opposite sides of an animal. In such cases the asymmetry probably does not change markedly in repeated reactions. But in cases like the one cited, where the stronger response is first on one side and then on the other, the balance which is disturbed must be a physiological one. We may assume that the processes of metabolism, or the previous reactions, or in short any of the factors which Jennings regards as effective in altering the 'physiological states,' have produced a change in the relative abundance or instability of the photosensitive chemicals of the receptive areas. Such an assumption is not unreasonable in view of the well known experiments of Loeb on the modifiability of light reactions by the use of chemicals; and the inducing by similar means of a light

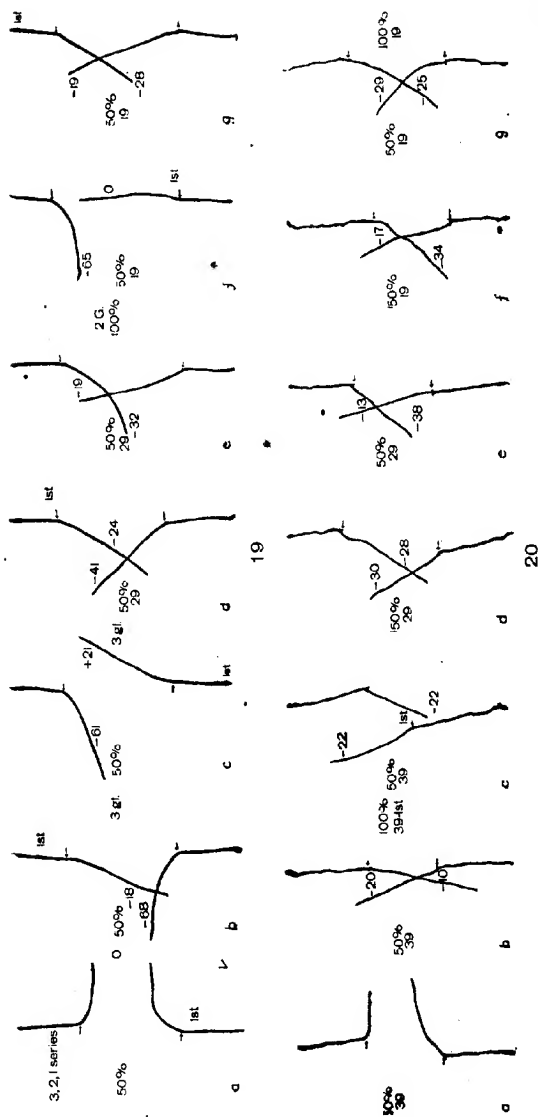


Fig. 19 Twelve trails made at 50 per cent difference by a larva which responded symmetrically throughout. Total deflection 288°; average angular deflection 24°; a, the test trails; b to f, trails made under the influence of the opposed lights.

Fig. 20 Twelve trails made at 50 per cent difference by a larva which reacted with varying unbalance of response. Total deflection 307°; average angular deflection 25.5°; a, the test trails; b to f, trails made under the influence of the opposed lights.

NOTE.—The letters, a, b, etc., designating separate records are placed at the extreme left lower corner of the record.

response in an animal which was normally indifferent to light, as recorded by Moore ('13).

The cases of natural and artificial asymmetry of susceptibility to light are not the only indications that the light receptive system operates on the musculature bilaterally. The deflection of symmetrically responding larvae toward the weaker light is in every way comparable with the deflection of asymmetrical larvae toward their less sensitive side when the illumination of both sides is equal. In one case the unbalanced factor of the response is external and in the other case it is internal. Moreover, it is possible to balance the internal asymmetry of the photosensitive areas by subjecting them to a corresponding inequality of illumination from without. For example, suppose, when a larva is subjected to equal lights from opposite sides, it deflects 5° toward the left. By referring to the curve of figure 15, it will be seen that, with asymmetry balanced out, a deflection of 5° is produced by a difference of 15 per cent between the opposed lights. With the left-hand light 15 per cent stronger than the right, the same larva crawls straight down between the lights like a symmetrically responsive larva crawling under the influence of equal lights.

The physiological asymmetry has been corrected by a difference of 15 per cent in the bilateral stimulus. Thus we can not only demonstrate that certain individuals do not have a perfect bilateral balance of sensitiveness but we can measure the amount of the asymmetry and correct it by applying bilateral light stimuli of a corresponding difference in intensity.

The evidence that symmetrically located sensitive areas operate bilaterally on the musculature may be summarized as follows: (1) When the lights acting on the opposite sides of the larva are equal, the larva orients so that its median plane is at right angles to the line connecting the sources of light. (2) When the opposing lights are unequal, a deflection toward the weaker light appears. (3) Certain larvae are asymmetrical in their response, deflecting toward the less sensitive side when subjected to equal bilateral illumination. (4) The blackening of one side of the sensitive region produces a deflection toward

the side artificially made less sensitive. (5) Asymmetry of sensitiveness may be balanced by a corresponding inequality of the stimuli acting on opposite sides of the animal.

There seems to be no explanation for the response of the blowfly larva to opposed lights other than the assumption that symmetrical sensitive areas operate on the musculature of the two sides of the animal in proportion to the stimulation received. We do not know precisely what mechanism is concerned in the reaction, nor even what it is that is 'balanced' in the receptors. There are, however, certain general lines on which such a mechanism must be based. If the angle of orientation under opposed beams of light is such that the stimulation of the opposite sides is equalized, the receptive mechanism must be of such a nature that varying the axial position of the animal produces changes in the relative amount of stimulation received on opposite sensitive areas. Otherwise there would be no cause for the animal to assume a definite angle of orientation for each intensity difference between opposed beams of light. This equalization cannot be accomplished by a median sensitive area unless we assume that the area operates differentially on opposite sides of the median line, an assumption which throws us back again to bilaterality. Nor can it be accomplished by bilaterally located sensitive areas that are parallel to each other, nor by fixed eyes so placed that the tangents to the eyes at the optical axes are parallel. This can be made clear by a diagram such as figure 21. The heavy black lines represent light-sensitive areas of, let us say, 1 sq. cm. of surface. In figure 21, *a*, the sensitive surfaces, being perpendicular to the rays of light, intercept 1 sq. cm. of the light as represented by their projection on a plane at right angles to the rays of light (double lines of fig. 21, *a*). In figure 21, *b*, the planes have been rotated through an angle of about 45 degrees. This cuts down the amount of light falling on each sensitive surface. But since the surfaces are parallel, their projection on a plane at right angles to the rays of light will be the same, and the amount of light falling on the two sensitive surfaces will still be equal. The case illustrated by the diagram, figure 21, *c*, is essentially like that of figure 21, *b*. The retinas will receive light proportional to that which falls

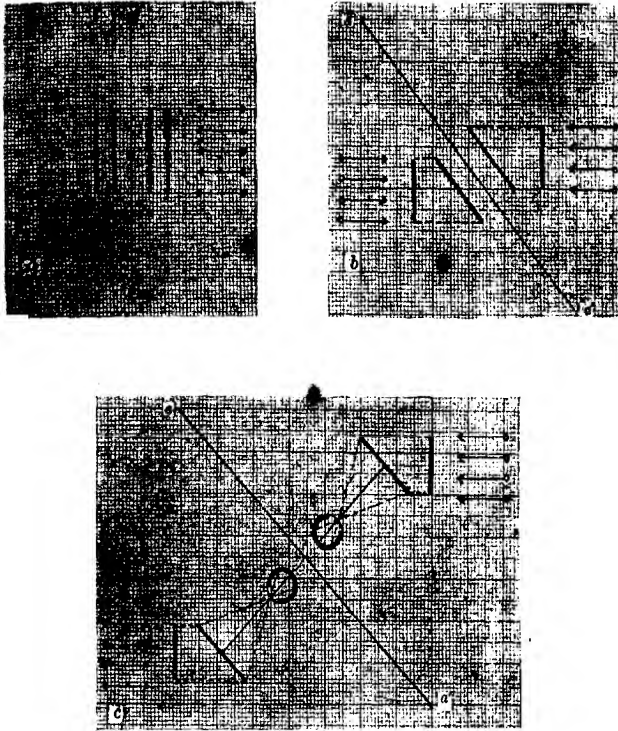


Fig. 21 A diagram showing that changes in the axial position of an organism with parallel sensory areas, or eyes with tangents at the optical axes parallel, do not change the relative amount of light intercepted by the sensory areas. In this figure, the lights, indicated by arrows, are assumed to be equal in intensity and opposite in direction.

on a plane paratangential to their optical axes at the focal distance of the dioptric apparatus. If these paratangents are parallel, rotation of the axes of the system will not change the relative amount of light intercepted by these planes. If, however, we assume that the sensitive surfaces are inclined at an angle to each other, the case is different. Figure 22 is a diagram constructed to show the conditions set up by rotation when the

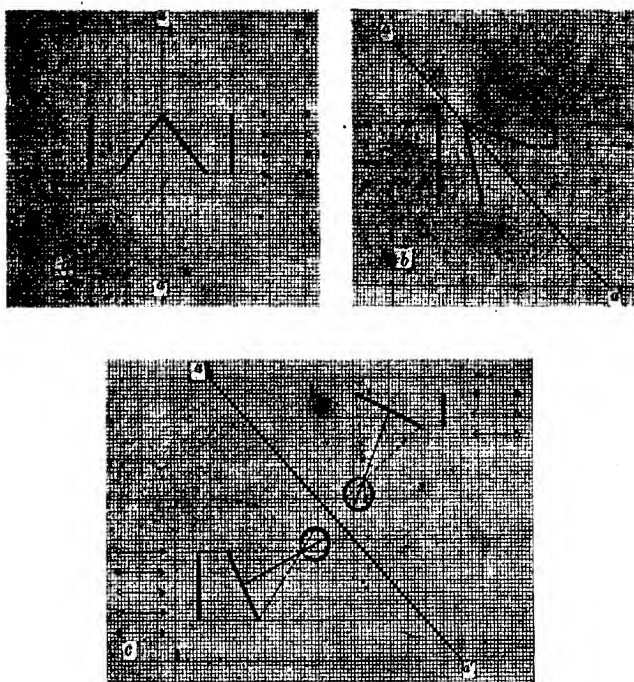


Fig. 22 A diagram showing that changes in the axial position of an organism with non-parallel sensory areas, or eyes with tangents at the optical axes non-parallel, change the relative amount of light intercepted by the sensory areas of opposite sides. Lights same as in figure 21.

sensitive surfaces are not parallel. In figure 22, *a*, the opposite surfaces intercept equal amounts of light because their angles of inclination are equal. In figure 22, *b*, the left area lies nearly at right angles to the rays of light and its projection is equal to its entire area, while the right side is inclined so that its projection is much less than its area. Consequently the amount of light acting on the bilateral photosensitive areas is different. In figure 22, *c*, the case is similar to that of 22, *b*. The amount of light received on the retina may not be exactly that operating on the paratangential plane at the focal distance of the eye, but it will be proportional to it.

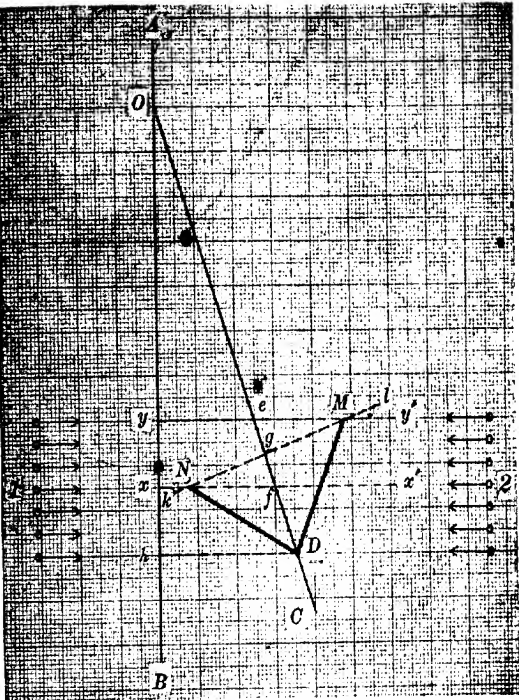


Fig. 23 Geometrical construction to show that when the sensitive surfaces are inclined to each other, a change in the axial position of the animal may equalize the light energy operating on them, although the intensity in the opposite sides of the field is unequal. The arrows indicate the direction, the numbers, the intensity of the lights; for construction and proof, see text.

We may assume, therefore, that the power to balance unequal bilateral stimulation, by a change in the position of the axis of the body depends on the inclination to each other of the photosensitive surfaces. The mechanism is simple and effective, and the explanation given fits the facts so far as they are known.

If this assumption be true, it should be possible to compute the angle of sensitive surfaces for an animal when we know its angle of orientation under varying inequalities of illumination. This angle has been computed for the blowfly larva, using the 'angular

deflections' already ascertained. The magnitude of the angle may bear no direct relation to the actual angle at which the sensitive areas are located in the body of the animal, because of the many factors which may modify the direction of the rays before they fall on the sensitive surfaces. The significant test of the hypothesis would be the constancy of the angle when computed from experimental data obtained under varying conditions.

The method of constructing such an angle is shown in figure 23, in which the opposing lights are assumed to be of a two-to-one ratio of intensity. The line AB is drawn perpendicular to the direction of the rays of light. On the line AB , construct angle BOC equal to the actual average angular deflection of the larvae at a two-to-one ratio of lights (p. 245). The problem now resolves itself into the construction of an angle about OC as a bisector, which shall be of such a magnitude that equal distances on its opposite sides shall have projections on the line AB of the ratio of two to one.

Construction: From a point D on the line OC draw Dh perpendicular to AB . Lay off on AB distances hx and hy , such that $hy = 2hx$. From x and y erect lines perpendicular to AB , they will intersect OC at f and e respectively. Bisect the line ef , and at its middle point, g , construct a line kl perpendicular to OC . From the point of intersection of kl and yy' (M), draw a line to D . From the intersection of kl and xx' (N), draw a line to D .

The angle MDN is the desired angle

Proof: $eg = gf$ (construction)

Angle $egM = \text{angle } fgN$ (construction)

Angle $Meg = \text{angle } Nfg$ (alternate int. angles of parallel lines, yy' and xx' being parallel by construction)

Therefore triangle $Meg = \text{triangle } Nfg$ (side and two adjacent angles being equal)

$Ng = gM$ (similar sides of equal triangles)

$gD = gD$ (identical)

Therefore triangle $NgD = \text{triangle } Mgd$ (rt. triangles, altitude and base equal)

Therefore angle $gDM = \text{angle } gDN$ and side $DM = \text{side } DN$.

Now by construction hx is the projection of DN on AB and ky the projection of MD on AB , and by construction $hy = 2hx$

This fulfils all the conditions of construction

The equal lines MD and DN represent equal bilateral sensitive areas inclined to each other at such an angle, MDN , that the surface represented by MD intercepts an area of light twice as great as the surface represented by DN , its projection on the perpendicular to the light rays being twice as great ($hy = 2hx$). But the light falling on DN is of twice the intensity of the light falling on DM , so that the total amount of light received by each of the equal areas is the same.

By this method of construction, the average angle of sensitiveness was computed for four intensity differences, using as a basis the angular deflection of the larvae as determined by experiment. The angles are shown in figure 24. The magnitude of the angles is almost identical in all four cases. This angle, I would emphasize again, probably does not represent the actual position of the sensitive surfaces in the larva.⁹ There are too many modifying factors intervening between the direction of the rays of light in the field and the angle of incidence of the light on the sensitive areas to permit of basing any conclusions directly on the magnitude of the computed angle, for the structural peculiarities of the dioptric apparatus, or of the tissue overlying the sensitive area would change its value to a large extent. However, the computed angle would be constant in a given animal, and the angle of the sensitive surfaces would be equal to K times the computed angle where K may be defined as the 'structural constant' of the animal.

An interesting problem in connection with theories of orientation is whether light operates as "a constant directive stimulus" or whether changes in the intensity are the main cause of stimulation. Without entering into a detailed discussion, some experimental evidence bearing on this question may be presented.

⁹ The organs concerned with light reception in the blowfly larva have not as yet been identified. Pouchet ('72) concluded that the two pairs of cones on the maxillal segment were not the light sensitive organs, and suggests that the imaginal discs of the adult eyes may function in the larva. Certain unpublished experiments of my own confirm his exclusion of the anterior cones. I have not been able, however, to obtain any positive evidence as to the organs concerned.

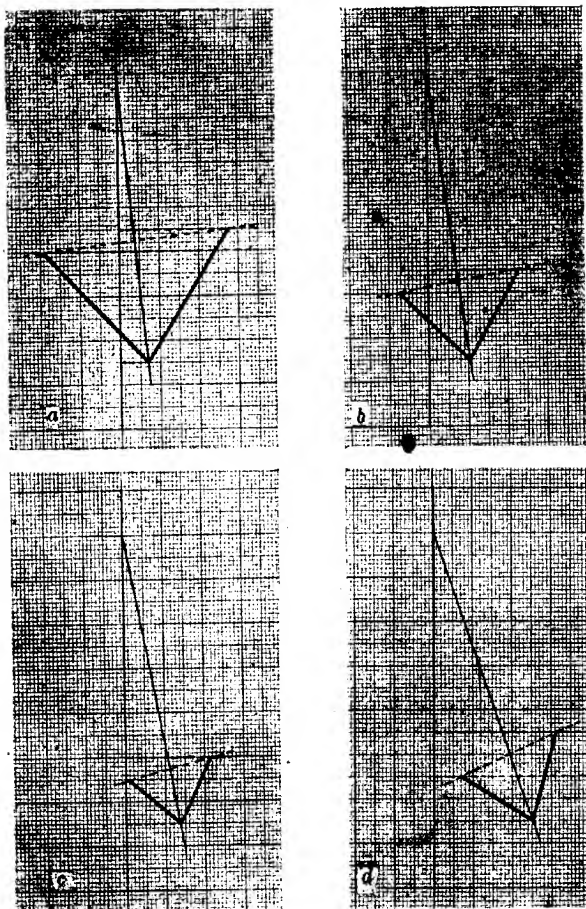


Fig. 24 Diagrams constructed after the method shown in figure 23; *a*, for 16½ per cent difference, constructed angle of sensitive surfaces = 82 degrees; *b*, for 25 per cent difference, constructed angle = 83 degrees; *c*, for 33½ per cent difference, constructed angle = 83 degrees; *d*, for 50 per cent difference, constructed angle = 82 degrees.

Recently Ewald ('13) has described experiments which prove that the Bunsen-Roscoe law holds for the response to light in the eye of *Daphnia*, as Loeb has maintained it should hold in animal light reactions (Ewald '13, p. 236):

This law states that in a light reaction the effect is proportional to the simple product of intensity and time. It was first proved to be true for the formation of hydrochloric acid from chlorine and hydrogen and for the blackening of silver chloride under the influence of light. Later it was found to apply to the phototropic curvature (Fröschel, Blaauw) of plants, as well as the human eye, though within rather narrow limits (Bloch, Charpentier).

If the law holds for the light reactions of photosensitive animals, intensity must of course operate as a constant stimulus.

Ewald found that the eye of *Daphnia* assumes "a definite normal position with regard to light" and that if while the animal remains fixed, the eye is subjected to lights from two sources, it takes up a definite axial position depending on the relative intensity of the lights. "In order to test the energy law, it is necessary to combine different light intensities with different times of exposure. If the product of time and intensity . . . is the same, the eye will always give the same reaction" (p. 236). This was proven experimentally by observing the position of the eye when subjected to one constant light and one light that could be varied at will. The variable light could be taken either from a constant low intensity source or through the apertures of a rotating sector wheel. An instantaneous shift from a slow, steady light to an intense intermittent light, delivering the same amount of light energy per second, caused no change in the position of the eye. If a sector wheel is used, giving too long or too short exposures to equalize the light, a change in the axial position of the eye appeared when the difference was greater than 10 per cent.¹⁰ "These observations prove that for the eye movements of *Daphnia* the energy law holds within the limits of accuracy characteristic of the reaction" (Ewald '13, p. 237).

¹⁰ In these experiments, the speed of the sector wheel was about 30 revolutions per second. With the reduction of the speed to 10 revolutions per second, a reaction was in some cases obtained when the change was made from steady to intermittent light.

Before the appearance of this paper, I had made a somewhat similar experiment on the blowfly larva, with the same results that Ewald obtained for *Daphnia*. Using the apparatus described on page 220, one of the beams of light was cut down by a diaphragm and the other by an episcotister, so that the light coming from one side was a steady beam of low intensity, and that from the opposite side an intermittent beam in which bright flashes alternated with darkness. The apertures in the sector wheel were adjusted so that the amount of light from each source was equal for a unit time. It has already been established that when the larvae are subjected to equal steady beams of light from opposite directions, the aggregate response is almost precisely at right angles to the line connecting the sources of light. The average angular deflection of 200 trails at equality (p. 240) was only 0.09° , when the degrees represented a distance of but 1.5 mm. If the Bunsen-Roscoe law holds for the phototactic response of the larvae, they should orient perpendicularly to the rays of light when subjected to the action of steady and intermittent lights of equal energy per second. The experimental results based on 136 trails made under these conditions show an average angular deflection of but 0.07° from the perpendicular.¹¹ These results seem to show that in the blowfly larva the phototactic reaction follows the Bunsen-Roscoe energy law.

Mast ('11, p. 234) says:

There is no conclusive evidence, except perhaps in animals with image forming eyes, showing that light acts continuously as a directive stimulus, that symmetrically located sides are continuously stimulated. . . . (p. 235). Light no doubt acts on organisms without a change of intensity much as constant temperature does, making them more or less active and inducing changes in the sense of orientation; but there is no conclusive evidence showing that light acting thus ever functions in the process of orientation.

Abrupt changes of intensity no doubt cause stimulation and are very effective in orientation under certain conditions¹² but they are not, as Mast maintains, the only ways in which light

¹¹ In these experiments the number of light impulses was 115 per second.

¹² See orientation of blowfly larva to single light, pp. 252 to 272.

operates to produce orientation, for our experiments on the blow-fly, to test the Bunsen-Roscoe law, clearly show that light of a constant intensity both stimulates and plays an important part in orientation.

Holmes and McGraw ('13) in experiments on insects have come to the same conclusion in regard to the effect of constant intensity ('13, p. 373):

It is not possible we believe to construe phototaxis entirely in terms of differential sensibility. Responses to the shock of transition, whether in the direction of an increase or a decrease of stimulation, may play a part in the orientation of many forms but the continuous stimulating influence of light appears to be in several cases at least the factor of major importance.

Bancroft's ('13) work, in which he showed not only that there was a distinct reaction to constant intensity present in *Euglena* but that it was largely the reaction to constant intensity which determined its orientation, shows the untenability of Mast's sweeping statement in one of the forms on which Mast himself worked.

The facts established concerning orientation in the larva of the blowfly may be summarized as follows:

1. When a larva is subjected to a single light, the changes of position, as it swings its head from side to side in the manner characteristic of locomotion and orientation, produce changes in the intensity of the light acting on the sensitive anterior end of the animal, due in a large measure to the shadow cast by its own body.
2. An abrupt change in the intensity of the light acting on the sensitive surfaces produces a reflex toward a "physiologically definite side"—the side on which the muscles are passively stretched.
3. Repetition of this reflex automatically checks motion toward the light.
4. In orientation to lights from two sources, the side to side swinging of the head does not produce changes in the effective intensity of the light on the anterior end as a whole.

5. Orientation to light from two sources depends on the relative amount of stimulation received by symmetrically located sensitive areas. This is shown by: perpendicular orientation to equal lights; deflection toward the weaker of two unequal lights; circus movements when one side of the sensitive areas is blackened; the presence of a natural asymmetry of response which may be counterbalanced by a corresponding inequality in bilateral stimulation.

6. An arrangement of bilateral sensitive areas may be postulated whereby stimulation on opposite sides of the animal may be equalized by a change of axial position. This arrangement accords with the facts so far as they are known.

7. The phototactic response of the blowfly larva depends to a large extent on the stimulating effect of constant intensity. The reaction to light of constant intensity follows the Bunsen-Roscoe law.

c. Analysis of factors involved in phototaxis. In the preliminary discussion of the relation of directive to non-directive light reactions, the interpretation was advanced that phototaxis had been evolved from photokinesis by the development, in connection with the latter, of certain factors which modify the action of the light on the organism, or indirectly distribute its effects. In the following section, an attempt has been made to analyze more closely certain of the factors involved in photokinesis and phototaxis, and to ascertain, as far as possible, their relative effectiveness.

The distribution of the stimulus in the field and on the sensitive surfaces of the animal, to a large extent determines the nature of the response. In a field uniformly illuminated from above there is, so far as we know, but one type of reaction, undirected activity which is maintained till muscular fatigue ensues or 'acclimatization' results. But if the field is not of uniform intensity and there are regions in it where stimulation does not take place, the organisms will sooner or later gather in those regions simply because they are not stimulated enough to move away from them. Under either mode of illumination, the reaction of the animal is of the photokinetic type; the condition which determines whether or not the aggregation takes place, lies not in the

reacting animal but in the distribution of the stimulus in the field. The term 'trial and error,' or better 'method of trial' may, I believe, be more appropriately applied to the method of aggregation by the kinetic reaction than to a method of orientation. The 'varied movements' of the locomotion of forms like *Stentor* or the blowfly larva take place whether or not the animals are stimulated by light. Though they undoubtedly play a part in orientation under some circumstances, it is by no means certain that they are essential to the attainment of orientation. In cases like that of a blowfly larva subjected to opposed beams of light, they probably play a very insignificant part in orienting the animal. It does not seem logical, therefore, to characterize the orientation of such forms as orientation by the method of trial, for even though there are some aspects of orientation that might be 'explained' on this basis, there are others which certainly cannot be so explained. An interpretation of orientation, to be acceptable, must accord not only with the details of a special case, but with all the varying details appearing under all the different manifestations of the phenomenon.

In the case of aggregation by the kinetic reaction, light causes the undirected locomotion which carries the animal into various environmental conditions. The movements initiated by light persist until 'selection' is accomplished with the cessation of stimulation, in other words, until the animal 'happens' to move into a non-photokinetic area. Such a reaction may be termed 'automatic distribution.'

A significant fact in connection with the operation of a uniformly distributed stimulus is that animals, such as the blowfly larva, which respond phototactically to horizontal light show a simple kinetic response when subjected to uniform illumination from above. The reason for this is the fact that under such conditions changes of axial position do not produce any difference in the relative amount of stimulation received by the sensitive areas. There is no basis for orientation. This establishes, as one of the critical factors in a directive response, a distribution of the stimulus such that a change in axial position on the part of the animal involves a change in the distribution or intensity

of the stimulus on the sensitive surfaces. For brevity, we may call a stimulus which fulfils these conditions a directive stimulus and one which does not, a non-directive stimulus.

Not all animals that are sensitive to light respond by orientation when they are subjected to the action of directive light. There must, therefore, be a second critical factor, a factor inherent in the responding organism. In the case of the blowfly larva, it was pointed out that orientation might be explained as a result of differential stimulation of bilateral sensitive surfaces inclined to each other at an angle, and the proportional transmission of that stimulation to the bilateral musculature. The explanation in this form will not apply to the case of an animal, like *Stentor*, which has but a single sensitive area. The two organisms, however, have in common a factor which I believe is a fundamental one in all directive reactions, a definite response to stimulation which is proportional to the intensity of the stimulus. In the blowfly larva there are bilateral sensitive areas, the stimulation of which produces a reaction bilaterally proportional to the amount of stimulation. In *Stentor* the spiral path of locomotion makes the single sensitive area perform the functions of two. There is a definite response to stimulation in the form of a swerving toward the aboral pole, and the amount of swerving is proportional to the stimulus. The operation of the response first on one side and then on the other brings about the orientation of the animal. Each animal has a method of reacting which is dependent on its structural peculiarities, but the animals respond in a definite and consistent way to stimulation by light, and both give a reaction proportional to the intensity of the stimulus.

When an animal does not respond to a directive light by orientation but merely by undirected activity, it is because the second critical factor of phototaxis is not present, the animal has no mechanism for producing a definitely directed response proportional to the intensity of the stimulus. We may assume that even in forms which do not respond directly, stimulation is proportional to the intensity of the stimulus, since the experimental evidence clearly indicates that stimulation, in its final

analysis is a chemical phenomenon and would follow the laws governing chemical reactions. The failure to respond directly is probably due, therefore, either to the presence of receptors of such a nature that changes of axial position do not affect the relative amount of stimulation received on opposite sides of the animal (fig. 21), or to the indefinite transmission of the stimulus from the end organs to the locomotor apparatus.

There are then two factors which must be present to produce phototaxis, one of these is resident in the stimulating agent, the other is resident in the organism. A directive stimulant may be ineffectual in producing orientation because of the absence in the reacting organism of a mechanism capable of directive response; similarly an animal with a perfectly developed directive mechanism may fail to orient because of the absence of directive distribution of the stimulating agent. The matter may be summarized thus:

Non-directive stimulant + indefinite response	= photokinesis
Non-directive stimulant + definite response	
proportional to the intensity of the stimulant	= photokinesis
Directive stimulant + indefinite response	= photokinesis
Directive stimulant + definite response pro-	
portional to stimulus	= phototaxis

The idea of a constantly acting stimulus which produces a reaction proportional to its intensity was one of the fundamental conceptions on which Loeb based his tropism theory. The fact that the theory as he advanced it postulated bilaterality in the responding organism has led to its abandonment by many authors who have studied the asymmetrically sensitive Protozoa. There is no doubt that in its original form the tropism theory does not apply to the orientation of asymmetrical organisms. The proportionality of the reaction to the intensity of the stimulant, within the range of normal physiological response, is however in my opinion the essential basis of any reaction involving definite orientation. Its method of expression varies in accordance with the structural peculiarities of the reacting animal, but the underlying phenomena are nevertheless in all cases essentially the same. If we include under phototaxis any reaction which involves a

definite axial orientation with reference to light, the tropism may be regarded as a special case of phototaxis in which the response depends on bilaterally placed sensitive areas.

The analysis of the factors involved in orientation as it has been presented here has been stripped of many details and limitations because I believe it has more value when stated broadly enough to allow details to be added as our knowledge of the subject increases.

I wish to express my indebtedness to Prof. G. H. Parker, under whose supervision this work was done, for his interest and helpful criticism, and to Dr. E. L. Mark for the facilities of the Zoological Laboratory and for the many courtesies shown me during my work there. I also wish to express the deepest gratitude to my father, who first aroused my interest in biology and whose criticism and encouragement have been a constant help.

SUMMARY AND CONCLUSIONS

1. The wide range of individual variability in the response of the blowfly larva to light renders a study based on untested animals of little value as a basis for comparing the effects of different experimental conditions.

2. To obtain reliable data for a comparative study of light reactions, it is necessary to establish a 'standardization test' and to make use of those individuals only, that show a uniform degree of sensitiveness.

3. To measure accurately the reaction to light in terms of definite physical units, the larvae have been subjected to the simultaneous action of opposed horizontal beams of light of known intensity, and the response measured, in degrees, on the resulting angular deflection of the trail.

4. When the opposing lights were of equal intensity, the average trail of the standardized larvae was within 0.09° of the perpendicular to the line connecting the sources of light.

5. When the opposing lights were unequal, the 'average trail' showed a deflection toward the weaker light. The amount of the

deflection was definite and constant, within the limits of experimental error, for a given intensity difference between the lights.

6. Using the average deflections obtained under each one of a graded series of intensity differences, a curve of the response to differential bilateral stimulations may be constructed. The deflection increases regularly with the increase of intensity differences.

7. The curve of response may be applied as a means of measurement to other experimental data obtained under similar conditions.

8. In orientation to a single horizontal light:

a. The changes of position due to the side to side swinging of the head characteristic of locomotion produce changes in the intensity of the light acting on the sensitive anterior end of the larva, due in a large measure to the shadow cast by the animal's own body.

b. An abrupt change in the intensity of the light acting on the sensitive surfaces produces a reflex toward a 'physiologically definite side,' the side on which the muscles are passively stretched.

c. Repetition of this reflex automatically checks motion toward the light.

9. In orientation to horizontal beams of light from two sources:

a. The side-to-side swinging of the head does not produce changes in the effective intensity of the light on the anterior end *as a whole*.

b. The attainment of orientation depends on the relative amount of stimulation received by symmetrically located sensory areas.

c. An arrangement of bilateral sensitive areas may be postulated whereby bilateral stimulation may be equalized by a change of axial position. This arrangement accords with the facts, so far as they are known.

10. The phototactic response of the blowfly larva depends, to a large extent, on the stimulating effect of constant light in-

tensity. The reaction to light of constant intensity follows the Bunsen-Roscoe Law.

11. The evolution of phototaxis is the result of the development, in connection with photokinesis, of certain factors which modify the action of light on the organism, or indirectly distribute its effects.

12. The critical factors of phototaxis are:

a. A distribution of the stimulant in the field such that a change in axial position on the part of the animal involves a change in the distribution, or intensity, of the stimulant acting on the animal or on its sensitive surfaces.

b. The presence, within the organism of a mechanism adapted to the reception of differential stimulation and a transmitting and motor apparatus that produces definite locomotor movements proportional to the intensity of the stimulation.

13. The 'response factor' may be present in the form of a bilateral mechanism, or in the form of a unilateral mechanism that reacts to both sides of the environment because of a rotational method of locomotion.

14. If we include under phototaxis any reaction which involves a definite axial orientation with reference to light, the tropism may be regarded as a special form of phototaxis, in which the response depends on the bilateral structure of the mechanism of response.

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THE BEHAVIOR OF THE EPIDERMIS OF AMPHIBIANS WHEN CULTIVATED OUTSIDE THE BODY

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SEVEN FIGURES (ONE PLATE)

In a previous paper¹ I have described the formation of strands and sheets ectodermic epithelium from pieces of amphibian larvae which were cultivated outside the body in lymph or plasma. It was shown that these extensions of epithelium were due to an amoeboid activity on the part of the cells at the edge of the extending mass of tissue. This year when opportunity for working on amphibian embryos and larvae was again presented, further observations and experiments on the behavior of the epithelial cells led to the discovery of several additional facts, as well as to the confirmation of the conclusions already drawn. Where not otherwise stated the observations here described were made on the larvae of *Diemyctylus torosus*. The behavior of the epidermis from tadpoles of *Rana* and *Hyla* proved to be essentially the same as in the species mentioned. Studies were also made on the epithelial outgrowths from tissues of the adult frog.

Pieces of embryos were cultivated as before in lymph or plasma from the adult. It is very difficult to employ plasma on account of the rapid coagulation of the blood. Lymph has the practical drawback that in most amphibians a very small quantity is available while in the frog it varies so greatly that no uniform results could be obtained with it. Lymph tends to become too watery for use a short time after the frog is dead, and only a few drops of a usable quality can be obtained from any one animal. Epithelial cells do not wander out to any considerable

¹ Holmes, S. J. Behavior of ectodermic epithelium of tadpoles when cultivated in plasma. Univ. of Calif. Pubs. in Zool., vol. 11, 155, 1913.

extent unless they are in a medium of more or less firm consistency. When the lymph does not form a coagulation about the tissue no outgrowth occurs, although the cells may remain alive and the cilia upon them keep beating for weeks. In Ringer's solution epithelium from both embryos and adults will remain alive for a long time, but no outgrowth as a rule takes place. As so many cultures put up in frog's lymph proved unsuccessful the attempt was made to find a substitute for coagulum of blood and lymph which would elicit the same sort of thigmotactic reaction from the epithelial cells. After several experiments which proved to be failures, it was found that a mixture of Grüber's nutrient gelatin and blood serum afforded a culture medium that was very suitable for the purpose in hand. The proportions of the substances finally decided upon were about equal parts of serum and a two per cent solution of the gelatin. The gelatin solution was made and sterilized by boiling. In obtaining the serum, blood was drawn from the heart into a small glass tube and the clot removed. Then the gelatin warmed sufficiently to become fluid was mixed with an equal quantity of serum and centrifuged to remove the blood corpuscles, and the clear liquid was then transferred to another receptacle. The mixture thus formed becomes quite fluid when but slightly warmed and remains fluid for an hour or more at ordinary temperatures. One can work with it therefore without undue haste, and should the supply become solidified in the course of making preparations of the tissues it can be made fluid again by a slight degree of warmth. The same fluid may be preserved for several days if kept free from infection. I have found tissue to thrive in it after it had been prepared for several days as well as in mixtures freshly made. The ease with which this medium can be made and used greatly facilitates making preparation of tissues, and the results obtained by its use are also more uniform than those obtained by the use of plasma or lymph.

The ectoderm from pieces of young tadpoles grows out in this medium with great rapidity. In twenty-four hours the new growth may exceed the area of the introduced tissue and in

forty-eight hours it may be five or six times as great. Usually the ectoderm extends in the form of broad sheets, but sometimes narrow strands are seen which often branch, and occasionally some of the branches may meet and fuse with those of other strands. In many cases most of the epithelium had migrated away from the implanted piece, but the latter was in all cases covered by a thin layer of this tissue. It is common for outgrowths to appear both in contact with the cover slip and with the lower surface of the hanging drop, and they are about as frequent in the one situation as in the other (figs. 1 and 2). During the period of active extension the advancing epithelium is always furnished with an amoeboid border of very clear protoplasm as has been described by Harrison.² This border varies much in width and is often so exceedingly thin and transparent that it is very difficult to follow its course. The very fine processes that are sent forth are mostly in close contact with the substratum, and a number of observations make it evident that they possess considerable adhesiveness. The conclusion reached in my previous paper that the epithelial extensions are due to the amoeboid activity of their hyaline margin is confirmed by the discovery of several additional facts.

It was found that the epithelial membranes possess a remarkable degree of contractility. With the application of a stimulus a large epithelial extension may shrivel up to about one-tenth its original area. Often a very broad extension several times the area of the tissue from which it came may contract to a very narrow fringe, giving one the impression that to a large extent it went back to its original situation. The contraction starting at any one point may be seen to spread to surrounding areas until finally the whole mass may be involved. At other times only a small part of the sheet of ectoderm may draw back. In either case the margin of the retracted ectoderm becomes much thicker and rounded, the amoeboid processes disappearing. If the amoeboid margin is watched carefully in an epithelial extension that is just beginning to contract the

² Harrison, R. G. The outgrowth of nerve fiber as a mode of protoplasmic movement. *Jour. Exp. Zool.*, vol. 9, 787-848. 1910.

amoeboid processes may be seen to give way suddenly as if they could no longer resist the tension of the cells behind. The free margin then becomes thickened and rounded, and the cells take on a quite different form. When one part of the hyaline border gives way the adjacent parts follow; the retraction, however, may soon stop, or it may spread widely according to various circumstances. At the beginning of the contraction the pseudopods may be stretched out considerably, as the cells behind tend to draw away, before they become loosened from their attachment.

A characteristic of these epithelial outgrowths quite as striking as their extreme contractility is their extraordinary sensitiveness to slight stimulations. Bringing the slides from a cool place to the stage of the microscope where the temperature is a few degrees higher usually causes a retraction of some part of the margin of an epithelial outgrowth. The process of contraction was observed a great many times, and in fact it usually happens to a certain extent whenever slides are brought from a cool place for examination. It is probably the transition to a warmer environment rather than the higher temperature per se that causes the contraction, because preparations kept at room temperature form equally great extensions of epithelium, and in many cases the sheets of ectoderm which become contracted when exposed to a higher temperature subsequently extended again while the higher temperature remained unchanged. Contraction of any part of the sheet of ectoderm may be initiated by placing the point of a warm needle above the region in question.

That the cells are responsive to a mechanical stimulus was shown by touching the margin of the extension with a fine glass rod. Local contractions uniformly followed. Whenever a preparation was washed in Ringer's solution preparatory to furnishing it with a new supply of nutrient gelatine, the epithelial extension shrivelled up to a small fraction of its previous dimensions. By placing a drop of Ringer's solution upon the preparation the epithelium is caused to contract as soon as the solution diffuses into contact with it. Whether the contraction is due to a slight osmotic effect of the Ringer's solution, or to the stimulating influences of the salts is uncertain.

In several cases a part of the epithelial outgrowth of a piece of tissue was cut off and transferred to another drop of the culture medium. The act of cutting causes a violent contraction of the epithelial outgrowths, and the operation usually has to be done quickly to be successful. A broad and very thin sheet of cells may shrivel up, after it is cut off, into a small rounded mass that has very little resemblance to its previous condition. Subsequently these isolated pieces may spread out as widely as before.

The application of an unfavorable degree of heat causes a contraction of the epithelium quite aside from any stimulating influence of a change of temperature. Often the areas of epithelium may be broken up in this way into isolated masses of cells or even into individual cells. The influence of heat on epithelium is very similar therefore to its effect on the blastomeres of a dividing egg. The cells or cell masses tend to become rounded up and inert. Light has very little effect on the epithelial cells. If the heat rays are filtered out, epithelium may be exposed for hours to the most intense light without manifesting any evident reaction.

In general one may say that epithelial cells, like so many free organisms, respond to various unfavorable influences by contraction. When kept for several days in the same culture medium the epithelial cells show a tendency toward rounding up. This often results in the rupture of strands of cells, or the breaking up of sheets of cells into isolated masses. Under favorable conditions epithelial cells rarely isolate themselves from the general mass. It is probably the accumulation of products of excretion that causes the contraction of cell masses which are kept too long in the same medium. The breaking up occurs much more quickly in cultures kept at room temperature than in those kept in a cooler place, owing probably to the more rapid metabolism and the greater accumulation of toxic products. That it is not the injurious influence of room temperature alone that causes the contraction of the cells is shown by the fact that by washing the contracted masses in Ringer's solution and giving them a fresh supply of the culture medium, the epithelial cells may again form extensions at ordinary room temperature.

Cultures may be kept alive for a long time at room temperature, but they require more frequent washing and change of medium. Extensions of cells occur more rapidly at room temperature than when the cultures are kept in an ice box.

While it is clear that extensions of ectoderm are mainly due to the outwandering of cells, a certain amount of cell division is also found to occur. Division figures were not infrequently observed several days after the preparations were made. In two cases a division figure first seen in the prophase was watched continuously through the entire process of mitosis. The individual chromosomes could be distinctly seen and as many as fifteen could be readily counted, although the actual number is greater. The arrangement of the chromosomes in the equatorial plate, their pulling asunder, and the formation of the two daughter nuclei could be easily followed. The chromosomes appeared as V-shaped rods, and during the anaphase the open end of the V's were directed towards the middle of the division figure. Cleavage of the cytoplasm was completed soon after the telophase of the nuclear division. The whole process of mitosis was completed in less than three hours.

Mitoses become rarer the longer the tissue is kept in a given supply of culture medium. In one preparation from the larva of *Diemyctylus* mitotic figures were common four days after the tissue was isolated, and some were found on the seventh day, but none later. Changing the tissue to a fresh supply of culture medium, however, may cause cell division to be resumed. One preparation which had been subcultured several times showed numerous division figures fifty days after it had been removed from the body of the animal. Two days after its last transfer into fresh culture medium it showed over twenty-five mitotic figures. One of these was watched through into the telophase when the cell body could be distinctly seen to constrict into two separate cells. I have never seen mitotic figures so abundant as in this piece of epithelium which had been kept for fifty days in an artificial medium. The washing in Ringer's solution and its transfer to a fresh supply of fluid had apparently given the tissue a new lease of life.

As stated in my previous paper, extensions of epithelium often give strong evidence of amitotic nuclear division. Further observation not only furnished additional evidence of the same phenomenon, but revealed some of the conditions by which amitotic division is induced. Newly extended sheets of ectoderm present little or no indication of amitotic division. The nuclei of the cells are round or oval and rarely present any indentation of their outline. With epithelial outgrowths which have been kept for a week or more in the same culture medium indications of amitosis are more frequent. Instances of two, three or four nuclei in a cell are common (fig. 3), and in some cases as many as eight nuclei were seen in a single cell. No clear indications of cell division following the division of the nucleus were observed. In many cases the amount of nuclear material in relation to the cytoplasm was obviously increased to a very considerable extent. In all cases where numerous nuclei were seen in a cell there was a considerable amount of yolk present, although cells with very little yolk remaining in them frequently had two nuclei, but rarely more.

While evidence of amitotic nuclear division occurred abundantly in many preparations kept without a change of medium, other preparations made of the same material but changed every few days to a fresh medium showed no indication of amitosis, although they were kept much longer than the preparations in which the medium was unchanged. The appearance of indications of amitosis very frequently goes along with signs of diminished activity, such as the rounding up of cells, the disintegration of certain cells in the culture, and the general inactivity of the epithelial tissue. It is a commonly received doctrine that amitosis occurs most frequently in cells of declining vitality. Its association in cultures of epithelium with life under unfavorable conditions lends a certain support to this view.

Most of the epithelial outgrowths observed showed cells of somewhat different types. The ordinary cells of pavement epithelium (fig. C), as soon as most of the yolk disappears, consist mainly of a clear, rather homogeneous protoplasm, more or less granular in the vicinity of the nucleus. A small amount of

yolk in the form of tiny spherules may persist until a late larval stage, even in the ectoderm of the tail and dorsal side of the body, while it is fairly abundant in the cells of the ventral side of the abdomen. As the cells become older and the yolk is gradually used up they tend to become relatively broad and thin, and to increase in transparency. Certain cells in early larval development become distinctly alveolar in structure (fig. 5). Such cells occur either singly or in small groups scattered about among the cells with homogeneous protoplasm. The alveoli are of various sizes in the same cell. These cells resemble and probably correspond to the Leydig's cells which have been described from the epidermis of several amphibians. I have observed the same type of cells scattered about in the epidermis of the young larvae of *Diemyctylus* in much the same way as they commonly appear in epithelial extensions in vitro. In the young larvae of Triton, Salamandra and Siredon, Maurer³ describes the Leydig's cells as scattered about singly among other cells of the deeper epidermis. On account of their vacuolated protoplasm these cells are considered as glandular in function, but they take no part in the formation of the cutaneous glands of later development. It is the appearance of the alveolar contents of the Leydig's cells that has given rise to the common opinion that they are mucous glands, but the cells have no direct connection with the exterior, and are usually covered with one or more layers of ordinary epithelium. In the preparations of epithelium in vitro various intergradations were found between ordinary epithelial cells and cells of the vacuolated type.

Occasionally also cells with granular contents occur in the epithelial outgrowths (fig. 4). The material forming the granules has a different appearance from the yolk, and probably represents the accumulated product of some sort of secretion.

As we have mentioned before, preparations may be kept alive for a considerable period if they are changed to a fresh culture medium. No especial attempt was made to find how long tissues could be maintained alive, but some of my preparations of epithelium were living over three months after implantation. One

³ Maurer, F. Die Epidermis und ihre Abkömmlinge.

cross section taken from the tail of a young *Diemyetilus* larva before it hatched from the jelly was put up in the middle of January. In a few days the epithelium showed extensive outgrowths along the cover slip and lower surface of the hanging drop. On January 27 after it had begun to show signs of deterioration it was washed and transferred to fresh gelatin and serum. Growth was resumed, and the cells showed a healthy appearance. The piece was transferred to fresh medium on February 2, and again on February 18. On February 24 it was divided into two parts. Both pieces, although their epithelial extensions became much shrivelled up after the transfer into fresh culture medium, soon put out extensive sheets of epithelium. Both pieces were transferred on March 3, when the same contraction and subsequent extension were again observed. One piece was transferred once more on March 10, and lived about a month longer. It is represented in figure 10. The second piece which showed signs of diminished vitality in the rounding up and separation of many cells and cell masses was re-transferred on April 1, after which it became active and sent out extensive sheets of epithelial cells.

The histories of many other preparations were essentially the same as the foregoing. In all cases the tissue was washed in Ringer's solution for a half hour or more, and the old gelatin mass around it removed before being put into a new supply of nutrient medium. A few preparations were left in Ringer's solution for a day without any apparent harmful results.

Epithelium remained epithelium throughout the experiments. The changes occurring in it were comparable to those taking place in the epithelium on the body of the larva. There was a progressive loss of yolk spherules, which in the epithelium from the dorsal side of the larva resulted in the total disappearance of yolk from most of the cells. As the yolk disappeared the cells became thinner and more expanded. From cells originally alike in appearance there came to be differentiation into cells of the vacuolated type, granular cells, although the latter were comparatively rare, ciliated cells, and cells of typical pavement epithelium. Whether the cells had received a start toward differ-

entiation into these different types before their implantation or whether their differentiation was entirely initiated after their transfer is uncertain.

A few experiments were tried with pieces of skin from the adult frog. Many of these pieces failed to show any epithelial outgrowth, although they were kept apparently in a healthy condition for weeks. In some instances large extensions of epidermis were observed. The cells at the margin of the outgrowths showed the same thin hyaline border with its minute changing pseudopods, as is found in the preparations of amphibian larvae. The general character of the epithelial outgrowth, the method of its formation and its behavior in relation to solid surfaces and to various stimuli are practically the same as in the extensions of larval epithelium.

Since my previously cited paper on the movements of epithelium in tadpoles was published, two papers have appeared which deal with the same subject. One of these is an article by Oppel⁴ which is devoted entirely to the epithelium of the tadpoles of *Rana*; the other, written by Osowski,⁵ includes a study of the movements of epithelial cells in tadpoles and in the embryos of fishes and of birds. The methods employed by both authors in their work on tadpoles are much the same. Tails of young tadpoles were cut off near the base, placed in diluted Ringer's solution for different periods, and then fixed and studied in serial sections so as to follow the way in which the cut surface came to be covered with epithelium. The covering of the cut surface was also followed by Osowski by making observations from time to time on the living tissue. No evidence of mitotic division was observed by Osowski in the epithelial cells that were extending over the cut surface. Both authors conclude that the movements of epithelial cells are responsible for covering the wound and that the movements in question are not the result of pressure or tension to which the cells may be subjected, but a con-

⁴ Oppel, A. Demonstration der Epithelbewegung im Explantat von Froschlärven. *Anat. Anz.* Bd. 45, 173-185, 1913.

⁵ Osowski, H. E. Ueber aktive Zellenbewegung im Explantat von Wirbeltierembryonen. *Arch. f. Entw.-mech.*, Bd. 38, 547-583, 1914.

sequence of the activity of the cells themselves. Osowski, however, does not look upon the movements of the cells as amoeboid. The general conclusion to which he comes in regard to the movements of epithelium in the forms which he studied is:

Die Bewegung, die in den Explantaten zu Wundüberkleidung führte, gehört also zu der Rubrik der Selbstbewegung, und zwar zu den aktiven Massenbewegungen des Epithels, weil sie sich an einer zusammenhängenden Masse von Epithelzellen gemeinsam abspielt.

Vergegenwärtigen wir uns das im experimentellen Teil über den Vorgang der Wundbedeckung Gesagte und beachten wir die beigegeben Figuren, so wird man in der Bewegung alle Charakteristika der von Oppel so genannten "Epithelbewegung" wiederfinden: Sie vollzieht sich ohne Pseudopodienbildungen, die Zellen bewegen sich hinter — und nebeneinander sowie parallel zu der zu überkleidenden Oberfläche.

Osowski's failure to find pseudopodia is, I think, due to his study of preparations by means of serial sections, and to the fact that his studies of living material were made by observing the epithelium in contact with other parts of the implanted tissue. The pseudopods of epithelial cells of amphibian larvae are so short and so fine that it would scarcely be possible to detect them when they were extending upon other parts. Only when these epithelial cells become freed so that one can observe their free margin with high powers of the microscope is it possible to ascertain the real method of their movement. Apparently neither Osowski nor Oppel observed epithelium extending away from the implanted tissue. This was due doubtless to the employment of Ringer's solution, which does not evoke a thigmotactic reaction from the cells.

I am compelled therefore to dissent from the view that the movement of epithelium in amphibian larvae takes place according to the conception of "Massenbewegung" as described by Oppel⁶ and adopted by his co-worker Osowski. Study of epithelial cells under the favorable conditions of isolation where they are spread out on the under surface of a cover slip clearly shows the essentially amoeboid character of their movements.

⁶ Oppel, A. Causal-morphologische Zellenstudien. V Die aktive Epithelbewegung, ein Faktor beim Gestaltungs- und Erhaltungsgeschehen. Arch. f. Entw.-mech. Bd. 35, 371, 1912.

The behavior of epidermal epithelium *in vitro* serves to throw light on several features of the activities and functions of epidermis in its normal situation on the body. It is well known that abraded or cut surfaces are quickly covered by an epithelial layer. The chief factor in this process, as Oppel and Osowski have contended, is epithelial migration and not the growth or the mere passive movement of epithelial cells. Surfaces may be covered with little or no cell multiplication, but simply by the wandering of epithelium. The cells of the epidermis have an inordinate tendency to lateral spreading. They have a strong tendency to cling to their own kind of cells, and under normal conditions seldom become isolated from their neighbors. The adhesiveness of the newly formed hyaline pseudopods serves to give a point of attachment, and as the hyaline border advances the epidermal cells are pulled out more and more. Various observations have shown that the epithelial membranes formed were drawn out despite a certain contractility of their substance. The fact that as soon as the attachment of the pseudopods is broken the membrane draws back and begins to shrivel up indicates that the real seat of activity responsible for pulling out the sheets or strands of cells is the amoeboid movement of the advancing margin. The extending of this margin often causes the cells to become exceedingly thinned out. The membrane tends to creep out until the advancing margin comes into contact with other epithelial cells, thereby forming a continuous covering of the exposed surface.

The strong tendency of the epidermal cells to lateral spreading insures the formation of a continuous membrane; this is an important property in relation to several functions, and especially those related to osmosis. Studies made on the peritoneal cells of amphibians showed that the behavior of these cells has much in common with the behavior of the cells of the epidermis. There can be little doubt that the peculiarities of arrangement of the cells lining the body cavity, the endothelial cells of the blood vessels, and the cells lining various ducts and other passages depend upon certain features of cell behavior. It is quite feasible to study the characteristic features of the behavior of these cells by keeping them isolated *in vitro*. Considerable light

may be thrown on various morphogenetic processes in this way, but it is perhaps premature to attempt to speculate as to the value or promise of this method of analysis.

SUMMARY

The epidermis of amphibian larvae grows well in a mixture of equal parts of a two per cent gelatin solution and blood serum from the adult animal. Epidermis shows a marked thigmotaxis, and in hanging drop cultures extends away from the implanted tissue along the lower side of the cover slip and the lower surface of the drop of culture medium.

The extending mass of epithelium usually takes the form of a broad, thin sheet whose outer border is formed of very thin, clear protoplasm furnished with fine pseudopodia.

Sheets of extending epidermis are sensitive to slight stimulations, and show a remarkable degree of contractility, often drawing in to a small fraction of their previous superficial area.

Strong contractions of epidermis are evoked by thermal, chemical, osmotic or contact stimuli, but strong light has no appreciable influence on the epithelial cells. After contraction the epithelial mass may spread out as widely as before.

Epidermis from amphibian larvae may be kept alive for several months if it is transferred occasionally into fresh culture medium.

Mitotic cell divisions were observed in the living cells fifty days after implantation into the culture medium.

Amitotic nuclear divisions were found, especially in preparations that were kept for several weeks without a fresh supply of nutrient fluid.

Epidermal cells from embryos of *Diemyctylus* were seen to undergo differentiation in vitro into the different types of cells found in the epidermis of late larval stages.

Pieces of epidermis from the adult frog form extensions much like those from the larva, but growth is less rapid.

The extensions of the epidermis in both larval and adult forms are due to the amoeboid activity of the hyaline protoplasm along the margin of the extending mass.

PLATE 1

EXPLANATION OF FIGURES

1 Epithelial extensions from a piece of the tail of a *Diemyctylus* larva a week after implantation. The darker sheet of ectoderm represents the part extending in contact with the cover slip of the hanging drop culture; the lighter lower extension was in contact with the lower side of the drop. 1^a represents the original piece which was taken from a young larva before it had hatched from the jelly; it was quite opaque and showed no chromatophores which had become clearly differentiated a week later.

2 Epithelial extensions from a piece of tissue which had been cultivated for over two months, subdivided once, and subcultured several times. The dark central part represents the piece from which two large sheets of epidermis, an upper and a lower, have extended.

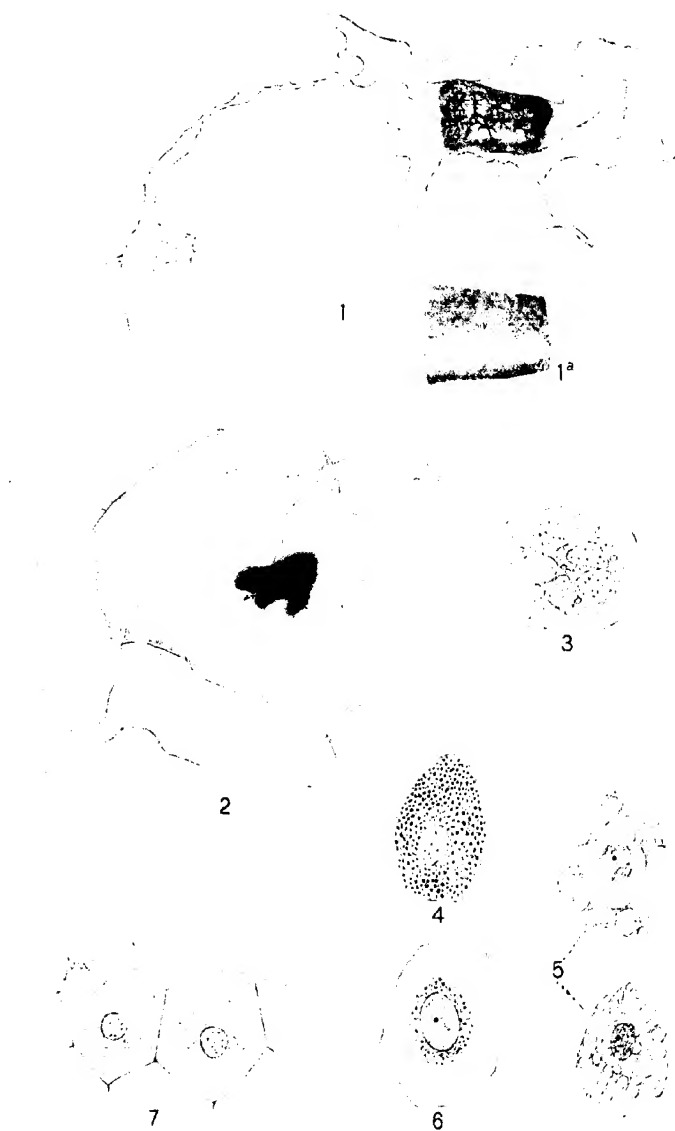
3 A multinucleated cell of epidermis.

4 Granular cell of the epidermis.

5 Two alveolar cells of the epidermis which differentiated *in vitro*.

6 Ordinary epidermal cell.

7 Epidermal cells from the edge of an advancing sheet of epidermis in contact with the cover slip. The thin hyaline border shows numerous fine pseudopods in contact with the glass.



EFFECT OF THYROID ON DIVISION RATE OF PARAMAECIUM

WALDO SHUMWAY

From the Zoological Laboratory, Columbia University

THREE FIGURES

INTRODUCTION

The remarkable results recently described (Gudernatsch '12, '13) of a thyroid diet upon the development of the tad-pole suggests interesting problems as to the effect of this substance upon the individual cell. In the following paper is given an account of the effects observed on the life history of *Paramaecium aurelia* when particles of thyroid were added to its diet. The experiments here described were carried on in the Zoölogical Laboratory at Columbia University and in the Marine Biological Laboratory at Woods Hole, under the direction of Prof. Gary N. Calkins, to whom the writer's thanks are due for encouragement and advice.

In studying the effects of chemicals on *Paramaecium*, many different indices have been employed by previous writers, especially chemotaxis, size, division-rate, and the phenomena exhibited by the gastric vacuoles. The rate of division has been chosen in these experiments as an index of the effects produced by the thyroid, as the investigations of Calkins, Woodruff and others have shown that it is very greatly affected by slight changes in the environment. Very recently Woodruff and Underhill have used this method in their series of investigations on "Protozoan Protoplasm as an Indicator of Pathological Changes" ('13-'14).

METHODS

All the experiments described in this paper were carried on upon a race of *Paramecium aurelia* descended from an individual isolated March 12, 1913 from a mass culture which had been maintained in the laboratory for several months. This race was hereafter maintained in four files¹ by daily isolations following the method described by Calkins ('02). The principal deviation was in the use of solid shallow watch glasses instead of depression slides. A daily record was kept of all the divisions which occurred in the history of the race, and these were averaged at the end of every five days to give the average division rate per day of the race for that period.

At the beginning of the experiments, two or three sister cells were isolated from each file, of which one was used to continue the control file while the others were used to start experimental files. In this way two or more lines of four files each were obtained of approximately the same protoplasm. Diagram 1 illustrates this method graphically. While one of the lines thus obtained was continued as the control line, the others were treated with thyroid or other substances as hereafter described. At the end of every five-day period, the division rates of the experimental lines were ascertained and compared with that of the control line for the same period.

The accompanying graph (diagram 2) shows the history of the control line (A) by five-day periods from the beginning of the experiment until December 20, 1913, when the line died out. The history of several of the experimental lines is indicated by dots and dashes.

The medium upon which the control line was kept was equivalent to one-half "standard hay infusion" (Jennings '10). It was prepared by boiling one gram of hay in one hundred cubic centimetres of tap water for ten minutes, filtering and allowing it to stand exposed to the air for twenty-four hours, when it was mixed

¹The term "file" is used to designate a single genetic series of *Paramecia* derived from an original individual by the process of daily isolating one of the cells produced by the divisions of the preceding isolated cell.

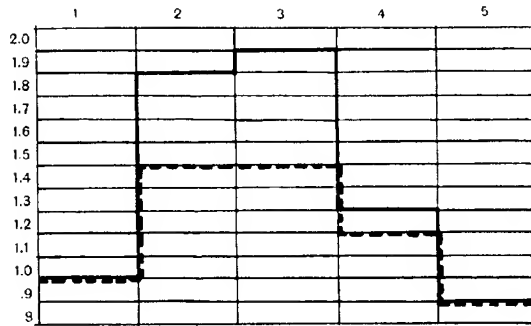


Diagram 1 To show ideal relations of sister lines of Paramecia for comparing difference in division rate produced by changes in environment.

———— = Control line = Sub-line for experiment

with an equal quantity of boiled tap water. The quantity used for each individual was four drops.

The experimental lines were treated with the regular control medium to which a small quantity of thyroid or thymus was added. Whenever possible, glands from the same animal were used. At first emulsions from fresh glands were employed, later Armour's dried preparations. No attempt was made to render the emulsions absolutely bacteria-free. In the following accounts of the experiments, the emulsions used will be referred to by the numbers under which they are here described.

I. Thyroid Emulsion I and Thymus Emulsion I, were prepared from the glands of ten rabbits killed and dissected April 6, kept in physiological salt solution twenty-four hours, macerated and mixed one part to two parts boiled tap water. These emulsions were kept in a refrigerator at about 10°C. and were used during period 7, in the proportion two drops to two drops 1-2 Standard Hay Infusion.

II. Thyroid Emulsion II and Thymus Emulsion II were prepared from the glands of twenty rats killed and dissected April 13, kept in Ringer's Salt Solution twelve hours, macerated, ground, and mixed one part to two parts boiled tap water. These emulsions were kept in the refrigerator, and used during period 8 in the proportion two drops to two drops 1-2 S.H.I., and during periods 9 to 12, in the proportion 1-4 drop to four drops 1-2 S.H.I.

III. Thyroid Emulsion III and Thymus Emulsion III were prepared from the glands of nine rabbits killed and dissected April 25, kept in physiological salt solution five hours, macerated, ground, and mixed one part to two parts boiled tap water. These emulsions were kept in the refrigerator, and used in Experiment 1, periods 13 to 15, and in Experiment 2, in the proportion 1-4 drop to four drops 1-2 S.H.I.

IV. Thyroid Emulsion IV and Thymus Emulsion IV were prepared from cow thyroids furnished me by the kindness of Dr. Gudernatsch. On June 28 these were macerated, ground and mixed one part to two parts boiled tap water and used in Experiments 3 and 4, during periods 23 and 24, in the proportion 1-4 drop to four drops 1-2 S.H.I.

V. This symbol will be used for preparations made daily from Armour's Thyroids U.S.P. (desiccated) and Armour's Thymus (desiccated) by shaking up 1.5 mg. in 2 cc. boiled tap water and adding 2 cc. 1-2 S.H.I. Four drops of these mixtures were used at each isolation.

EXPERIMENTS

Experiment I. April 9 to May 23, 1913.

Average Division Rate

PERIOD	A (CONTROL)	D (THYROID)	E (THYMUS)	F (THYROID-CONTROL)	LINE
7	1.50	2.70	0.55		
8	1.05	1.95	1.10		
9	1.00	2.90	1.10		
10	1.55	3.60		1.90	
11	1.45	2.80		1.40	
12	2.20	3.15		2.25	
13	0.95	2.15			
14	1.75	2.60			
15	1.00	2.40			

For this experiment two lines of four files each were derived from the control line (A), one of these (D) was kept in 1-2 S. H. I. to which a small quantity of thyroid (Thyroid I, II, III, see above) was added, the other (E), not shown on diagram 2, was

Diagram 2 Chart showing life history of line A, together with the differences produced in sub-lines treated with thyroid, some of which were thereafter returned to control conditions.

———— = Control line

..... = Lines treated with thyroid

- - - - - = Lines returned to control conditions after thyroid treatment

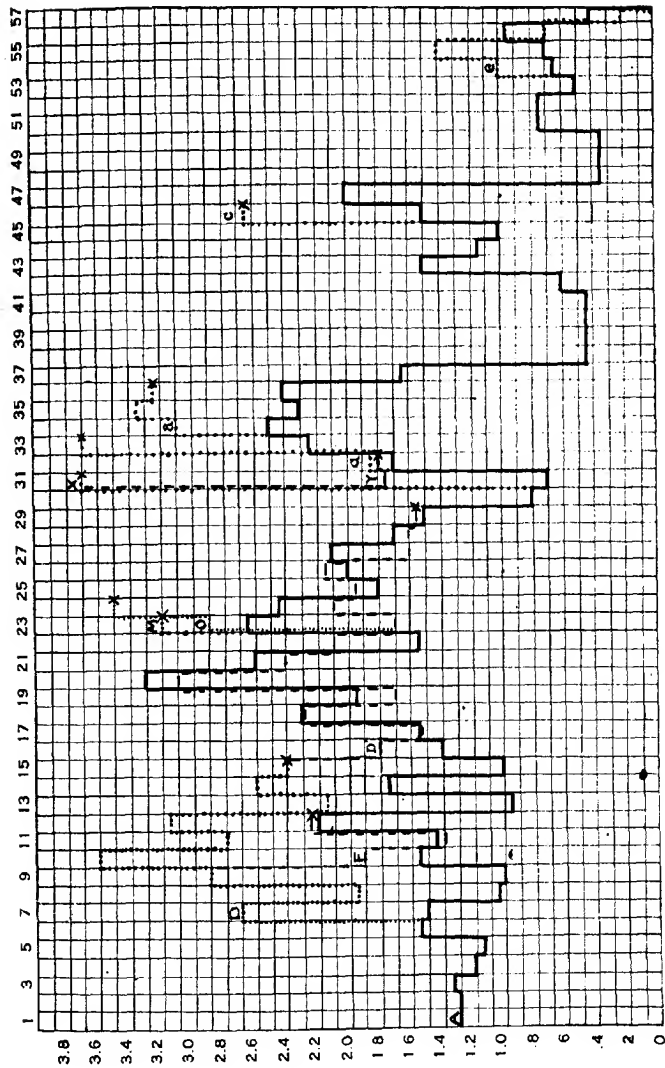


Diagram 2

treated in the same way except that thymus was substituted for thyroid. The table above shows the average division rate for each line during the periods in which the experiment was continued. As is seen, the average for D is always greater than for A, while E does not show this increase. The F line, started from the D line after fifteen days of thyroid treatment and kept thereafter in control medium, shows a return to the average rate of A.

Experiment II. April 24 to May 8, 1913.

PERIOD	Average Division Rate					LINE
	G (CONTROL)	H (THYROID)	J (THYMUS)	A (CONTROL)	D (THYROID)	
10	2.15	3.15	1.00	1.55	3.60	
11	1.20	2.80		1.45	2.80	
12	2.50	3.10		2.20	3.15	

This experiment was commenced during Experiment I to discover whether the effects observed in the thyroid treated individuals would be produced in other individuals of the same race. An individual was selected from the A line, and allowed to divide until there were twelve cells of the same generation. Four of these were allowed to continue in the control medium, forming line G, four were treated with Thyroid III (H), and four with Thymus III (J). The table shows the division rate for the three ensuing periods. For purposes of comparison, I have added the averages given by the control and thyroid lines of the preceding experiment for the same periods. This experiment confirms the results of Experiment I.

Experiment III. June 28 to July 2, 1913.

PERIOD	Average Division Rate			LINE
	A (CONTROL)	M (THYROID)	N (THYMUS)	
23	2.65	3.20	2.15	

This experiment was performed at Woods Hole. After the trip from New York and the change to Woods Hole tap water

the division rate as a rule averaged much higher than it had in the city. As my supply of Thymus and Thyroid IV was limited, and owing to the difficulty of keeping fresh tissue, this experiment was continued for one period only. It is sufficient however to show the typical thyroid increase. Compare the following experiment.

Experiment IV. June 28 to July 7, 1913.

PERIOD	Average Division Rate		LINE
	D' (CONTROL)	O (THYROID)	
23	1.70	2.90	
24	2.10	3.50	

This experiment was carried on at the same time as Experiment III. The control in this case was the D line (now known as D') which had been treated with thyroid for 9 periods and then maintained on a control medium since the close of Experiment I. While in this medium it had approximately given the same averages as A, as may be seen from diagram 2. The O line was derived from this and treated with Thyroid IV for two periods, during which, as may be seen from the table, it maintained a much higher rate of division.

Experiment V. August 7 to 21, 1913.

PERIOD	Average Division Rate				LINE
	A (CONTROL)	X (THYROID)	Y (THYROID-CONTROL)	Z (THYMUS)	
31	0.70	3.70	1.75		
32	1.70		1.80	1.80	
33	2.25		2.50	2.00	

At the time of this experiment the life-cycle shows an apparent depression period (diagram 2) and the race was reduced to two individuals. One of these was kept in the control medium to continue the A line, the other was treated with Armour's desiccated sheep thyroids (Thyroid V) to start the X line. After

one days thyroid treatment individuals were taken from the X line, returned to the control medium to initiate the Y line. The table shows the great increase produced by the thyroid in the D line and the subsequent return to normal in the Y line. The Z line derived from the Y line and treated with thymus, shows nothing significant.

Experiment VI. August 12 to 21, 1913.

PERIOD	<i>Average Division Rate</i>		LINE
	A (CONTROL)	a (THYROID)	
32	1.70	1.85	
33	2.25	3.70	

As the control line began to emerge from the depression period, another race (a) was derived from it and treated with Thyroid V as above. After one period in which no significant increase appeared, the thyroid treated line presented a high division rate, comparable with that shown in Experiment V.

Experiment VII. August 22 to September 5, 1913.

PERIOD	<i>Average Division Rate</i>			LINE
	A (CONTROL)	a' (THYROID)	d' (THYMUS)	
34	2.50	3.10	2.50	
35	2.30	3.25	2.00	
36	2.40	3.25	2.95	

This experiment is rendered more significant by the fact that instead of four files, eight files were maintained for each line. The table shows the significant increase in division rate exhibited by the thyroid treated line. Note also that in this as in other experiments where Thymus V was employed, that the dried thymus does not produce the decrease in division rate observed when the fresh glands were used. It may be said in passing that this decrease was due to the large number of deaths which appeared in those lines.

Experiment VIII. October 21 to 25, 1913.

PERIOD	Average Division Rate			LINE
	A (CONTROL)	c' (THYROID)	d' (THYMUS)	
46	1.50	2.65	1.30	

This experiment was performed after returning to New York from Woods Hole and an interval at which observations were made at irregular intervals (one to four days), periods 38-41 inclusive. At this time the Thyroid (V) still produced an increase in division rate.

Experiment IX. November 30 to December 19, 1913.

PERIOD	Average Division Rate			LINE
	A (CONTROL)	e' (THYROID)	f' (THYMUS)	
54	0.65	1.00	0.80	
55	0.70	1.40	1.15	
56	0.95	0.70	1.05	
57	0.40	0.20	0.15	

This experiment was carried on during the final depression period which carried off the race. The individual cells at this time gave evidence of their condition not only in lowered division rate but also in distortion, decreased size, more sluggish movements and the appearance of monstrosities. Attempts were made to save the race by changing the culture medium to beef extract, increasing the amount of medium per individual, treating with K_2HPO_4 , etc., but in spite of these efforts the last individual died on December 20. At this period the thyroid (V) did not produce any effect after the first two periods; in fact the thyroid treated individuals succumbed more rapidly than the control. The thymus treated individuals showed no significant differences.

DISCUSSION

In the experiments described above, we have seen that in every case, disregarding for the moment Experiment IX, the thyroid treated line has divided more rapidly than the control line from which it was descended. Thymus treated lines on the contrary, while apparently showing a decreased rate of division, have in reality given only negative results, for the lower rate of division was caused by a greater number of deaths. Lines returned to the control medium after several periods of thyroid treatment have then given the same average division rate as the control, while their sister lines under continuous thyroid treatment have continued at the higher rate of division.

The source of the glands used bears no relation to the effects produced. A typical thyroid increase was produced by preparations from fresh glands of rats, rabbits and cattle, and from dried glands of sheep. The stability of the agent is shown by the fact that emulsions have continued active for twenty-five days during which time putrefactive decomposition had commenced. The accompanying graph (diagram 3) shows the effect of two emulsions (Thyroid II and III) the one from rats, the other from rabbits, for five successive periods. It will be seen that the two curves are similar, although at this time I have not sufficient data to discuss the nature of the curve.

I was however unable to secure any positive results with filtered extracts, either from fresh or dried material. This is the reverse of the results of Nowikoff ('08) who has published the only other experiments on this question I have been able to discover in the literature. It might be well then to discuss this paper in some detail.

While studying the chemotaxis of *Paramaecium* to thyroid extract, Nowikoff observed that the individuals treated with thyroid seemed to divide more rapidly than those kept in hay infusion. He devised some experiments intended to demonstrate this increase in division rate: from these he concluded that the thyroid extract "exerts an intensive influence." Nowikoff used Merck's *Glandula Thyroidea sicc. pulv.* dissolved in

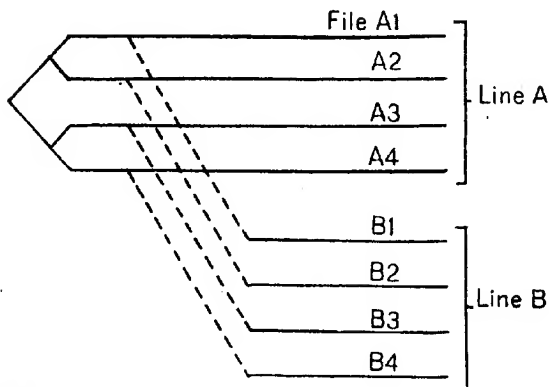


Diagram 3 Chart showing comparative effects by five day periods of Thyroid II and Thyroid III. The ordinates represent the increase of the thyroid average over the control average per period, the abscissae the periods covered.

———— = Thyroid II

..... = Thyroid III

distilled water, shaken and filtered two or three times through paper, in a one or two per cent solution. A number of individuals (one to three) were placed in a few drops of this extract on a slide; other individuals were placed in a similar quantity of hay infusion or other media. The preparations were covered with a cover slip and set aside in a moist chamber. Daily observations were made and showed larger numbers of individuals in the thyroid media than in the others.

This method of Nowikoff's does not seem, however, adapted to answer the question at issue. Woodruff's experiments ('11) upon the toxicity of the material excreted by *Paramecia* have shown that the division rate of individuals kept in the same medium for a number of days grows slower owing to the harmful effects of this excreted material. It is significant that in Nowikoff's experiments the *Paramecia* kept in hay infusion under these conditions died out in a few days, as we should expect. Then too the thyroid extract, kept in a moist and fairly warm chamber for periods up to a month, must have undergone appreciable chemical change. It is evident that the experiment is complicated by the introduction of a toxic, if not lethal, factor and by the lack of a uniform thyroid preparation.

Another source of error in these experiments lies in the absence of an attempt to secure closely related *Paramaecia* for the experiment, the necessity for which is shown by the great variability in reaction and viability possessed by different races and individuals of this form.

I have endeavored to calculate from Nowikoff's daily counts of individuals the number of divisions which took place, although as I have pointed out this is made difficult by our ignorance of the number of deaths which occurred. This calculation is shown below, and indicates that using the rate of division as an index, his results are inconclusive.

Calculated division rate for Nowikoff's Paramaecia treated with Thyroid, Hay Infusion, and Muscle

MEDIUM	DAYS										TOTAL
	1	2	3	4	5	6	7	8	9	10	
Hay Infusion.....	0	0	0	0							0
Thyroid.....	0	3	3	0	0						6
Thyroid.....	0	3	2	1	0						6
Muscle.....	0	2	1	0	2	0	0	0	1	0	6
Hay Infusion.....	0	0	3	1	0	0	0	1	0	0	5
Thyroid.....	3	3	1	0	0	0	0	0	0	0	7
Thyroid.....	2	1	0	3	0	0	0	1	0	0	7

Gudernatsch, in his second study of feeding experiments on tadpoles ('13), describes an experiment in which small pieces of thyroid gland were placed "in a small glass vial closed with gauze and allowed to stand upright, the open end being under the surface of the water." The typical rapid macroscopic differentiation normally produced by thyroid feeding was observed, and Gudernatsch concludes that "the product of the thyroid which caused the rapid differentiation must be soluble in water." The conditions of the experiment do not however preclude the possibility of minute particles of the thyroid escaping through the gauze and becoming food for the tadpoles. A definite conclusion can only be drawn from experiments conducted with carefully filtered preparations.

In order to secure a conclusive answer to the question whether *Paramaecia* actually ingested particles of thyroid, the writer performed the following experiment, suggested by the methods employed by Métalnikow ('12). Having stained some particles of Armour's desiccated thyroids with an alcoholic solution of Congo Red, and washed out the free color in distilled water, they were placed in a watch-glass of boiled tap water with several individuals of *Paramaecium caudatum*. It was possible to watch the formation in the *Paramaecia* of gastric vacuoles containing these prepared thyroid particles, and, following the cyclosis, to observe the change from acid to alkaline reaction shown by the Congo Red as described by Métalnikow. I have permanent preparations fixed with osmic acid vapor that show these thyroid filled vacuoles very clearly.

If then the *Paramaecia* can and do ingest these thyroid particles and if after ingestion the chemical reactions which are commonly accepted as indicating digestion are observed, does it not follow that the effects produced by the thyroid result only after ingestion and presumably digestion? Certainly this is in accord with Gudernatsch's results.

The individuals treated with thyroid were as a rule more active, and slightly smaller than the control individuals, and their protoplasm was more transparent. This would be the case if the process of metabolism were accelerated by the thyroid. The thymus treated individuals on the other hand were more sluggish, larger and darker than the control forms, these appearances apparently being due to large quantities of undigested food granules. The conclusion seems obvious that the effect of the thyroid is directly upon the metabolic activities of the cell.

With the effects of the thyroid upon growth and differentiation this investigation is obviously not concerned.

Returning now to Experiment IX we find the data presented more difficult to analyze. Here the thyroid does not increase the division rate at a time when the control line is losing the power of division and evidently dying out. Compare this with Experiment V, when the control line was also at a very low state of

vitality, but the thyroid treatment resulted in the greatest increase in division rate observed at any time.

The explanation, I believe, lies in the fact that in these two Experiments the *Paramecia* were at very different stages in their life history and that their protoplasmic make-ups were not comparable. I believe that in Experiment V a typical depression period (Calkins) or rhythm (Woodruff) was occurring, from which the race was able to recover without stimulation, while the thyroid was able to produce its usual effect. In Experiment IX however I suggest that we have a period of depression leading to "germinal death" such as carried off Calkins' race of *Paramecium caudatum*. Under this hypothesis we can understand how the thyroid was without effect in the final depression period, for if some element of the protoplasm necessary for digestion were lacking, the thyroid agent could not be released or act. While this is advanced only as an hypothesis, it is a significant fact that at the time when the race was nearing the end of its life cycle, the thyroid which had previously produced a significant increase in the rate of division with such absolute uniformity, presented no perceptible effect. Attention is also called to the fact that this race of aurelia died out in four hundred and twenty generations, while Woodruff's race of the same species has been maintained for more than four thousand generations, one more example of the great differences in vitality manifested by different races of *Paramecia*.

SUMMARY

Experiments have been carried on to demonstrate the effect of thyroid and thymus added to the diet of a pedigreed line of *Paramecium aurelia* during a life history of four hundred and twenty generations. These experiments have shown that the effect of the thyroid is to increase greatly the rate of division, except at the time when the line was nearing the close of its cycle; and further that the effect is not permanent after feeding with thyroid is stopped. Similar treatment with thymus gave negative results. It has been shown further that the *Paramecia* actually ingest and probably digest particles of thyroid.

From these experiments it has been suggested that the effect of the thyroid is primarily upon the metabolic activities of the cell, and further, that at the close of the life cycle some element of the protoplasm necessary to digestion is lacking, thus inhibiting the effect of the thyroid.

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APPENDIX

Daily records of divisions

Here are given the number of divisions per day for each file of every line described in Experiment 1 of this report (the other experiments are only summarized in the preceding pages). At the end of every five days the total number of divisions for each file for that period is given. The averages cited in the text are obtained by adding the totals of the files of each line and dividing by 20 (or the exact number of opportunities for division).

A letter and number enclosed in brackets are used to designate from what file an individual has been replaced after its loss from death or accident, e.g. (A2). The symbol ° is used to indicate that an individual died but was replaced by a sister individual from the last preceding division of the same file. In this case the number of divisions of the sister individual are credited to the file, so 1°. The symbol - indicates a death without replacement.

Experiment I

APRIL	9	10	11	12	13	(7)	14	15	16	17	18	(8)
A1	0	2	2	2	1	7	2	2	1	0	(A2)	5
A2	1	1	2	1	2	7	2	1	0	(A3)	1	4
A3	0	1	1	0	0	2	0	0	(A4)	1	1	2
A4	0	2	2	2	2	8	2	2	1	1	1	7
D1	(D3)	2	4	3	3	12	3	1	2	1	2	9
D2	(D4)	2	4	3	3	12	2	4	1	2	2	11
D3	2	2	3	3	3	13	2	3	0	1	2	8
D4	1	2	4	3	2	12	3	2	2	2	2	11
E1	0	2	1	2	(E2)	5	(A1)	(E3)	(E3)	1	2	3
E2	0	1	1	(E1)	2	4	(A1)	(E3)	1	(E3)	1	2
E3	0	(E1)	0	(E4)	—	0	(A2)	2	2	2	1	7
E4	0	1	(E1)	1	—	2	(A2)	(E3)	2	2	1	5

EFFECT OF THYROID ON DIVISION OF PARAMAECIUM 313

Experiment I (continued)

APRIL	19	20	21	22	23	(9)	24	25	26	27	28	(10)
A1	1	1	1	2	0	5	1	2	2	1	2	8
A2	1	1	1	2	1	6	1	2	1	1	2	7
A3	2	1	1	1	0	5	2	2	2	1	2	9
A4	1	1	2	0	0	4	0	(A3)	2	2	3	7
D1	4	3	2	3	2	14	4	4	3	3	0	14
D2	4	2	3	3	2	14	4	4	4	4	5	21
D3	3	3	3	3	3	15	1°	3	3	4	5	17
D4	3	4	2	3	3	15	3	4	4	1	5	20
E1	1°	1°	(E3)	2	4	F1	3	0	2	1	(F2)	6
E2	1°	1°	(E3)	3	5	F2	3	3	2	3	2	13
E3	1	1°	2	2	1	7	F3	2	2	3	2	10
E4	1°	1	(E3)	3	5	F4	2	2	1	3	1	9

APRIL	29	30	MAY 1	2	3	(11)	4	5	6	7	8	(12)
A1	1	1	1	2	2	7	3	2	1	3	1	10
A2	1	1	1	3	1	7	3	3	1	3	1	11
A3	1	(A4)	2	2	2	7	2	3	2	2	2	11
A4	1	1	2	2	2	8	3	3	1	3	2	12
D1	0	(D2)	4	3	4	11	4	3	2	4	2	15
D2	3	1	4	1	3	15	4	3	2	4	2	15
D3	3	2	3	4	3	15	5	3	3	3	2	16
D4	3	2	3	4	3	15	5	3	3	3	3	17
F1	1	0	2	2	2	7	4	3	1	3	1	12
F2	2	1	2	0	2	7	3	2	2	3	1	11
F3	2	1	1	1	2	7	2	2	2	3	1	10
F4	2	1	1	1	2	7	3	3	2	3	1	12

MAY	9	10	11	12	13	(13)	14	15	16	17	18	(14)
A1	2	0	(A2)	0	2	4	2	3	3	1	2	11
A2	1	0	1	0	2	4	2	0	1	2	2	7
A3	2	1	0	1	2	6	1	2	2	1	2	8
A4	1	1	0	1	2	5	1	2	3	1	2	9
D1	3	2	2	1	2°	10	3	3	3	2	2	13
D2	3	2	2	1	3	11	3	3	3	2	2	13
D3	3	2	1	2	3	11	3	3	3	2	2	13
D4	3	3	1	1	3	11	3	3	3	2	2	13

Experiment I (continued)

MAY	19	20	21	22	23	(15)
A1	1°	1	1	1	1	5
A2	2	0	1	1	1	5
A3	1	2	0	1	1	5
A4	2	0	1	1	1	5
D1	4	3	2	1	1	11
D2	4	3	2	2	2	13
D3	3	3	1	2	3	12
D4	1°	(13)	2	1	0	4

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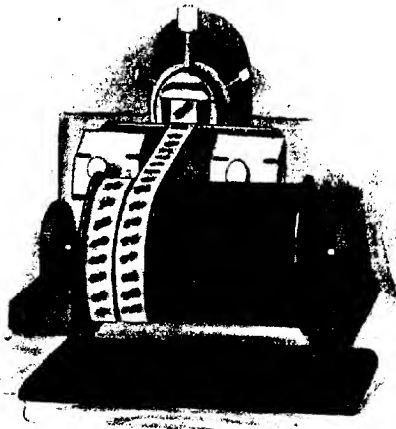
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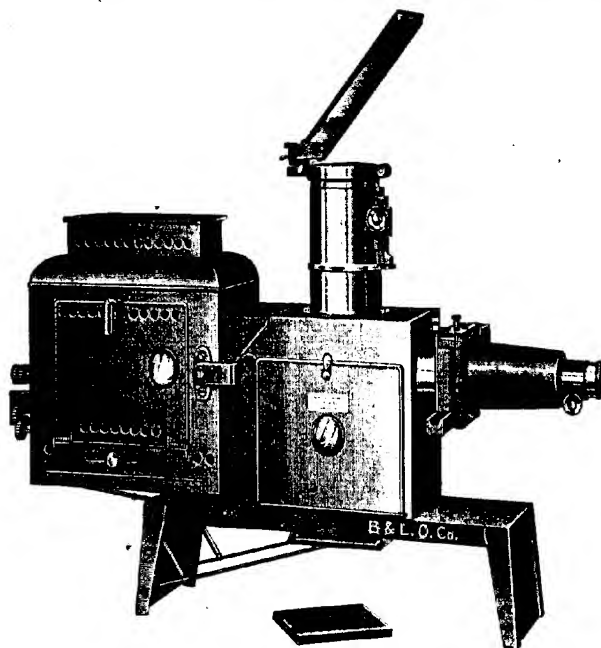
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A THIRD SEX-LINKED LETHAL FACTOR IN DROSOPHILA

T. H. MORGAN

Department of Zoölogy, Columbia University

THREE FIGURES

In an experiment (reported in the Jour. Exp. Zoöl., page 390, November, 1911) in which three sex-linked characters were involved, namely, white eyes, vermilion eyes and miniature wings, certain results were obtained involving double crossing-over. The more recent results indicate that double crossing-over was excessive in this experiment.¹ A white miniature male had been mated to a vermilion pink ('orange') female. The daughters were wild type (red, long) and the sons vermilion (long). The two sex-chromosomes of these daughters contain respectively the genes for white (w), red (V), and miniature (m); and for red (W), and vermilion (v), and long (M). All later work has shown the order of the genes here involved is white, vermilion, miniature, as shown in diagram 1.

On this basis, the results of the previous experiment in terms of cross-overs would be as follows:

	<i>Total</i>	<i>Per cent</i>
Non-cross-overs (vermilion and ver. pink males)	333	74.7
Single cross-overs between white and vermilion (min. and min. pink males).....	81	18.2
Single cross-overs between vermilion and min. (verm. min. and ver. min. pink males).....	11	2.4
Double cross-overs (wild type and pink males)...	21	4.7

Instead of giving fewer double cross-overs than singles, according to expectation, the number of doubles was twice that of singles, and for numbers as large as these this difference cannot be due to

¹ In the analysis on page 392 the large C in 'sperm no X' should be small c. This change adds eight classes to the white males and eliminates the same number from the colored classes. The expected numbers in the male classes at the top of page 393 should read downwards: 3-3-1-1-8-3-3-1-1-8.

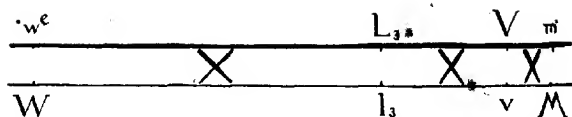


Diagram 1

chance. This experiment has been repeated, therefore, in small mass cultures and has given, in F_2 , the totals shown in table 1.

TABLE 1
Females

WILD TYPE	VERMILION	PINK	VERM. PINK
1428	1465	469	548

Males

VERMILION	VERM. PINK	MIN.	MIN. PINK	VERM. MIN.	VERM. MIN. PINK	WILD TYPE	PINK	WHITE	WHITE MIN.
501	161	251	84	14	35	7	0	314	1166

The females conform to expectation. The males show many irregularities that will be discussed in another connection. Classified as in the original experiment, the males give:

	<i>Totals</i>	<i>Per cent</i>
Non-cross-overs.....	662	63.8
Single cross-overs between white and vermilion..	335	31.8
Single cross-overs between verm. and minia- ture.....	49	4.7
Double cross-overs.....	7	0.7

The totals of these new results for the males approach very closely to expectation both for the amount of double crossing-over and also for the distances apart of the genes, despite other inequalities that appear in the results.

In the experiment as actually carried out a non-sex-linked gene, namely, the gene for pink eyes, is involved. The additional classes that are thus introduced are ignored in the preceding calculations in which pink and not-pink classes are combined. If we take pink into consideration the following relations will be found. The gene for pink lies in another chromosome, and since the F_1

female is heterozygous for pink every egg after the polar bodies are extruded will contain either the pink factor or its normal allelomorph. The male has two classes of sperm, viz., one with and the other without the pink factor. In all cases except white (which remains white in external appearance in either case) the combination gives two classes in the ratio of 3:1. The relation of pink to not-pink are everywhere normal except in the following case. The single cross-overs between vermilion and miniature are vermilion miniature (14) and vermilion miniature pink (35). Instead of the expected 3:1 ratio the relation is almost reversed. I can offer no explanation of the relative deficiency in the vermilion miniature class or the relative excess of the vermilion miniature pink.

We may assume that the double cross-over class included in white miniature is very small and negligible; if so, then the non-cross-over class white miniature should equal the sum of the vermilion and vermilion pink, which is 662. In fact, it is nearly double (1166) that number. This disturbance, as will now be shown, is due to a lethal factor present in the stock.

THE LOCATION OF LETHAL III

In the experiment, made as explained above to test double crossing-over, only about half as many males as females appeared in some of the cultures. This kind of result we have come to associate with a lethal factor; and further tests made with some of the F_2 females show, in fact, the presence of a new lethal. Table 2 gives individually the ten original mass cultures (p. 318).

Since the cultures were made in mass they can not be used to study lethal factors, for, the offspring may have arisen from both lethal-bearing and non-lethal-bearing females. The high sex-ratio was not noticed until the experiment was nearly finished, and then it was possible to obtain only a few females.² In three cases

² We have known that non-disjunction occurs in several of our stocks, notably in the white. It occurred in the mother in the experiment just described, for when vermilion pink females were mated to white miniature males there were produced:

Verm. ♀ 2	Red ♀ 62	Verm. ♂ 46	White ♂ 2
--------------	-------------	---------------	--------------

The two vermilion females and the two white males must have arisen through

TABLE 2

Females

	WILD TYPE	VERMILION	PINK	VERMILION + PINK
I.....	128	123	39	51
II.....	151	154	37	58
III.....	173	169	61	76
IV.....	86	89	37	36
V.....	250	231	51	81
VI.....	84	84	41	44
VII.....	90	93	32	33
VIII.....	94	96	28	31
IX.....	189	228	68	58
X.....	184	188	75	80
	1428	1465	469	548

Males

	VERM.	VERM. PINK	MIN.	MIN. PINK	VERM. MIN.	VERM. MIN. PINK	WILD TYPE	PINK	WHITE	WHITE MIN.
I.....	57	16	24	9	3	10			33	120
II.....	72	25	30	5	2				36	142
III.....	24	15	29	9	1		2		29	133
IV.....	26	4	10	2	1		2		16	37
V.....	103	30	46	17	2	11			51	193
VI.....	34	12	11	7		2	1		16	78
VII.....	16	5	12	5					13	66
VIII.....	35	4	17	10	1	7			17	74
IX.....	63	24	28	10	2	1	1		45	173
X.....	71	26	44	10	2	4	1		55	150
	501	161	251	84	14	35	7		314	1166

at least, viz., IV, VIII and X, the sex-ratios were again obtained from F_2 females. Further tests were made of the stock derived from the F_3 lethal females in order to locate the lethal or lethals present.

non-disjunction. One of the vermilion females was tested by breeding to a pink male. She gave:

Red ♀ 56	Pink ♀ 61	Verm. ♂ 43	Verm. pink ♂ 19	Red ♂ 2
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The two red males are again due to non-disjunction.

Vermilion females were out-crossed to club males. From those sets that gave a sex-ratio, daughters (red eyed) were mated singly to their vermillion-eyed brothers. About half of the females gave no sex-ratios, as follows:

● All females	Club ♂	Verm. ♂	Club verm. ♂	Wild type ♂
687	219	314	58	64

The cultures that gave sex-ratios follow (table 3):

TABLE 3				
ALL FEMALES	CLUB ♂	WILD TYPE ♂	CLUB VERM. ♂	VERM. ♂
146	53	3	1	
176	50	7	3	
145	34	10	2	1
121	36	8	5	
588	182	28	11	1

The smallest class of males is vermillion, which we assume therefore to be the double cross-over class. Since the lethal went in with the vermillion female one of the chromosomes of her daughter will contain the genes for lethal and for vermillion; and the homologous sex chromosome will contain the gene for club. The relation of the two chromosomes with the normal allelomorphs of these genes are shown in diagram 2.

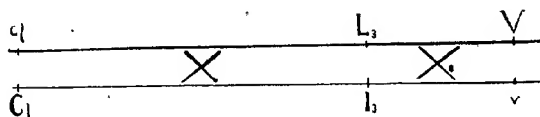


Diagram 2

In order that vermillion become the double cross-over class the lethal must be between club and vermillion, as in the diagram. The chromosome distances calculated from the results are as follows:

Club lethal III.....	13.0
Lethal III vermillion.....	5.4
Club vermillion.....	18.0

Other vermilion females were out-crossed to eosin miniature males. From the cultures that showed sex-ratios females were back-crossed singly to eosin miniature males. The back-cross cultures that gave sex-ratios are seen in table 4.

The results of the experiment confirm the location of the genes in diagram 1, as the following chromosomal distances show:

Eosin lethal III.....	20.2
Eosin vermilion.....	27.0
Eosin miniature.....	29.0
Lethal III vermilion.....	7.0
Lethal III miniature.....	9.3
Vermilion miniature.....	2.3

TABLE 4

Females

EOSIN MIN.	WILD TYPE	EOSIN	MINIATURE
78	104	33	24
90	89	36	33
82	84	37	45
101	77	31	30
105	97	31	43
127	86	35	33
62	62	29	13
60	53	36	38
58	51	24	30
763	703	292	289

Males

EOSIN MIN.	MINIATURE	EOSIN VERM.	EOSIN	VERM.	WILD TYPE
84	18	8	4		
101	20	6	2		
96	27	11	1	1	
95	27	3	3		
103	17	11	3		
128	16	13	2		
52	17	2	2		
76	31	10	3		
39	23	7	2	1	
68	26	8	4		
52	19	6	4		
44	24	6			
938	265	91	30	2	1

The small numbers in the first experiment gave a distance between lethal III and vermilion of 5.4; the second gave 7. The data of both experiments gave 6.8.

In a third case vermilion females (lethal-bearing) were crossed singly to vermilion miniature males and gave:

VERMILION ♀	VERM. MIN. ♀	VERM. MIN. ♂	VERMILION ♂
113	78	75	7
77	99	79	7
190	177	154	14

These results gave a distance of 9 units between the lethal involved and miniature and the lethal may lie either to the right or to the left of miniature. If to the left, the locus corresponds to lethal III and may be so taken.

A HIGH SEX-RATIO

A vermilion female (IXX^a) second generation from F₂ was bred to a vermilion male. Two of her vermilion daughters were bred (in the same culture)³ to two white vermilion brothers and gave a ratio of:

Vermilion ♀	Vermilion ♂
71	3

A remarkable high sex ratio is here met with. Many of the vermilion daughters were bred separately. A partial report of the results, namely, most of the cases in which the females were bred to eosin miniature males, is included in table 5.

Certain other daughters (of the 71) were bred to their (three) vermilion brothers and gave the results shown in table 6.

Still other daughters (of the 71) bred to eosin miniature males gave the results seen in table 7; where, in addition to the regular classes of females, there are some irregular classes that cannot be explained. These exceptional individuals appeared only in the

³ It is possible that the offspring came from only one of these females.

TABLE 5

WILD TYPE ♀	WHITE-EOSIN COMPOUND ♀	WHITE ♂	VERMILION ♂	PER CENT OF CROSS-OVERS
59	43	37	7	16
35	44	38	6	14
70	81	50	15	23
48	38	31	13	30
71	63	62	17	22
48	57	53	16	23
68	65	71	16	18
87	95	68	27	28
59	34	38	9	19
61	60	45	8	15
86	66	52	15	22
61	59	63	16	20
23	35	14	0	
70	62	60	11	15
43	60	50	10	17
40	53	30	10	25
51	49	33	9	21
52	51	23	4	15
53	51	40	4	11
1085	1066	858	213	

TABLE 6

VERMILION ♀	WHITE ♂	VERMILION ♂	PER CENT OF CROSS-OVERS
184*	78	19	19
62	35	5	12
79	28	4	13
25	8	3	27
83	20	4	17
56	21	5	19
489	190	40	

*Mass culture which is not included in curve on page 323.

TABLE 7

IRREGULAR ♀ ♀		REGULAR ♀ ♀				PER CENT OF CROSS-OVER
White ♀	Verm. ♀	Red ♀	White-eosin compound ♀	White ♂	Vermilion ♂	
	3	32	26	36	10	22
11	16	51	41	53	10	16
7	4	38	15	21	2	9
13	22	14	38	27	9	25

early hatches of the cultures.⁴ On the other hand, the proportion of males is the same as in the other cultures.

In the last columns of the three preceding tables the percentages of cross-overs between white and the lethal are given. If these are tabulated they show bimodal curves (diagram 3).

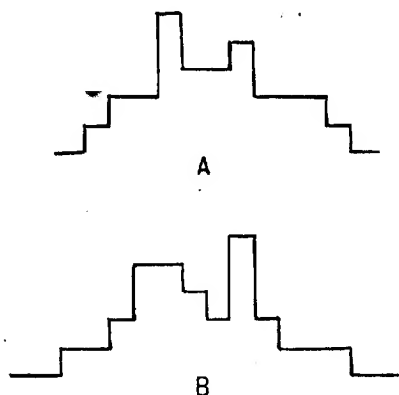


Diagram 3 Bimodal curves from the grouping of the linkage values from tables. A, grouped 9 + 10, 11 + 12, etc. B, grouped 8 + 9, 10 + 11, etc.

All the data taken together suggest the following interpretation. If an original female that gave rise to the 71:3 ratio had two lethal genes that are not allelomorphs, one in each of her sex chromosomes, all of her sons should perish except those few which resulted from crossing-over. The three males would be these cross-overs and indicate the amount of crossing-over that had taken place (provided their number is doubled, for the other equal class of cross-over males perished). Using the 71 females as the number of all the expected males and dividing this into 6 would give the percent of cross-overs, namely, 8 per cent.

In the second place, all the 71 daughters should be lethal-bearing, except 8 per cent corresponding to the males that survive. *In fact, all of 34 females tested were lethal.*

⁴ If the first-hatched daughter had been fertilized by the father before they were removed (as virgins!) this result can be explained. In the next generation no repetition of the occurrences was seen.

In the third place, the linkage values of these daughters should fall into two classes corresponding to the two lethals, and since the two lethals are only 8 units apart the curve representing the values should have two modes about 8 units apart. This result also seems to be realized, as shown in diagram 3.

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A GENE FOR THE FOURTH CHROMOSOME OF DROSOPHILA

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Drosophila ampelophila contains two pairs of long 'autosomes' (chromosomes other than sex-chromosomes) and one pair of minute 'autosomes'; there are, in addition, a pair of long sex-chromosomes alike in the female ('X's) but unlike in the male ('X' and 'Y').¹ Correspondingly, breeding tests of the numerous mutants of *Drosophila* have revealed a great group of genes (containing over thirty members) which are sex-linked in such a way that they are distributed, in the reduction division, to exactly the same cells as is the X-chromosome, and two other great groups (with about twenty members each) which are not sex-linked and so have been considered to follow in their distribution two of the pairs of autosomes (presumably the long ones). The genes in these three groups are therefore said to lie in Chromosomes I, II and III, respectively. The members of any group of genes are linked with one another; in the female the linkage is partial and is of the linear type to be expected on the basis of the chiasmatype theory of Janssens and Morgan; in the male the linkage is complete (no crossing-over). All the members of one group assort independently of the members of either of the other groups, in both sexes, as would be expected on the generally accepted postulate of the random assortment of non-homologous chromosomes in the reduction division.

¹ Two lines of recent cytological investigation, the one followed by Mr. C. W. Metz, the other by Mr. C. B. Bridges, have given evidence (not yet fully published) which goes far towards refuting the view suggested by Stevens that the so-called 'X' chromosome of *Drosophila* actually consists of a short autosome with the real X-chromosome joined to it and that the 'Y' chromosome represents this short autosome without any 'X' attached.

It will be seen that the above grouping of genes leaves one pair of autosomes (presumably the small ones) and the Y-chromosome without any genes to correspond. As regards the Y, it seems at first sight surprising that no mutations have been found involving genes which follow it in their distribution (i.e., genes which are always transmitted from father to son), for it is of about the same length as the X, in which over thirty genes are known. This fact would force us to one of three conclusions: (1) Either the genes connected with the Y-chromosome for some reason do not mutate; or (2) these mutations are all recessive to dominant normal allelomorphs present in X (in spite of the fact that mutations in X are not dominated, conversely, by allelomorphs in Y); or (3), as Mr. C. W. Metz first suggested, genes are degenerate or entirely absent in the Y-chromosome. The first conclusion is *a priori* unlikely; the third may now be supported by a number of considerations, which it will be of interest to discuss briefly in this connection, before proceeding to our account of the fourth chromosome.

In the first place, the Y-chromosome is known to vary greatly in size and number in closely related species of animals. Secondly, as Mr. C. B. Bridges has recently shown, a female occasionally, owing to an abnormal reduction division ('non-disjunction'), receives a Y-chromosome in addition to the two X's, yet such a female is indistinguishable from the ordinary form, which contains no Y. The Y-chromosome therefore either contains no genes or else only genes which are allelomorphic to those in X, but never dominant to them. This is proved also by the fact that mosaic flies sometimes develop, of which a part of the body is female but another part is male, owing to the accidental loss of one of the X-chromosomes in an embryonic cell-division. These male parts must have an X, but no Y, and yet they are indistinguishable from corresponding parts on real males which contain a Y. Furthermore, Mr. C. B. Bridges has obtained (again by 'non-disjunction') males which must have two Y's, yet these also show no peculiarities. It is therefore certain that Y either contains no genes at all which have an effect upon the individual or it contains genes allelomorphic to those in X. It can be proved, however, that if it contains allelomorphs to the

genes in X, these are not normal allelomorphs (i.e., the same kind as those present in the X of the wild fly) for they are recessive to mutant genes in X to which the normal genes in X are dominant, i.e., a male always manifests all the genes, mutant or normal, recessive or dominant, that are present in its single X-chromosome. In other words, if there be genes in Y allelomorphic to those in X, they are abnormal allelomorphs of those in X, and are always recessive to all genes in X. It is difficult to conceive why genes in Y should be recessive, on the one hand, to normal genes in X, in those cases where the mutant genes in X are dominant to the normal, and, on the other hand, to mutant genes in X, in those more frequent cases where the normal genes in X are dominant to the mutant, unless the genes in Y are mere 'absences' or nearly so.²

There is an *a priori* explanation for this lack of genes, or lack of dominant genes, in the Y-chromosome, an explanation in the development of which Dr. A. H. Sturtevant has coöperated with me. Owing to the fact that crossing-over never occurs in the male *Drosophila*, any mutation which originally occurred in Y remained in that chromosome and was never exchanged for a normal gene from X. Furthermore, these mutations in Y which were recessive would not have been subject to the action of natural selection for, since the normal gene in the X-chromosome will dominate over them, individuals containing them will not be abnormal. In the course of time, therefore, recessive changes in the Y-chromosome will tend to accumulate. If, now, we assume that mutations sometimes consist in losses³ of genes, a degeneration of the Y-chromosome (so far as its *genes* are concerned) would result.

² This might be otherwise explained on the very improbable hypothesis that all of the mutations which occurred in X were in a restricted part (the end?) of the chromosome, and that the Y lacked only this part of the chromosome and so appeared to contain genes recessive to all those in X which underwent mutation, although it actually contained normal genes in the other part of the chromosome, which never mutated. This is not quite equivalent, although nearly so, to the idea that X is attached to an autosome (see footnote 1).

³ By losses of genes are meant not necessarily their total bodily disappearance (losses of loci from the chromatin) but also any changes in them whereby they are rendered permanently inactive and incapable, under any circumstances, of exerting an influence on the organism.

This is because most losses of genes, if they occurred, would be recessive (i.e., one 'dose' of a factor usually has the same effect as two), as indicated by the fact that the one-X part of a mosaic is like the XX part in respect to all characters affected by X except the sex characters (provided the two X's of the XX part are alike). Now, if, as seems likely, recessive losses are more apt to occur than recessive additions of genes (since one 'dose' usually has the same effect as two) the Y-chromosome will gradually become functionless. On *a priori* grounds, we should expect such a fate, in any species, for the sex-chromosome peculiar to the heterozygous sex, if crossing-over never occurs between it and its homologue.⁴

These considerations will also explain the size difference which often exists between X and Y, the apparent unimportance of supernumerary Y-chromosomes in development, when these occur, and other irregularities of the Y-chromosome. Hitherto it has been a mystery why the difference merely in the sex factor or factors contained in the sex chromosomes should often be correlated with such a large difference between the two chromosomes, whereas differences in respect to other factors did not involve any visible size differences in the chromosomes concerned, which presumably contain hundreds of genes.

There is, therefore, all things considered, no cause for surprise in the fact that no mutations have occurred involving a group of genes transmitted only from father to son, and thus following in their distribution the Y-chromosome. The chief gap, if it may be so termed, then remaining in the parallelism between the configuration of the chromosomes and the distribution of genes in *Drosophila* has been due to the fact that no genes were found in a fourth independent group (a third independent non-sex-linked group) to correspond with the fact that there are three pairs of autosomes.

⁴ Conversely, where this chromosome appears degenerate or different genetically from its homologue (in addition to the difference in the sex factors) we should expect to find no crossing-over between the two sex-chromosomes in the heterozygous sex, i.e., complete, not partial, sex-linkage. This argument applies to all known cases of sex-linkage, for in all these cases a recessive mutant factor can manifest itself in the heterozygous sex, proving that the sex-chromosome peculiar to the heterozygous sex is different from the other sex-chromosomes in that it contains no dominant normal allelomorph of the mutant factor.

Supposing, however, that mutations are equally likely to occur at any locus in the chromatin, it could be explained as a result of pure chance that no mutations had as yet happened to lie in the restricted space of the small autosome. It was expected, nevertheless, that mutations in a fourth group would eventually be found, and such a mutation has now in fact arisen.

The new character is a recessive wing and leg abnormality, the wings being held out from the body but bent backwards near the base, and the metatarsal joint of the legs being frequently greatly shortened and thickened. The wing is also apt to be curved, with the dorsal surface convex, and shortened. The character varies somewhat, but there is very rarely any difficulty in distinguishing it from the normal form, unless the flies have been raised in very dry bottles. Drought therefore hinders the manifestation of this character, as it may also, and to a greater degree, in some way hinder the development of the character 'abnormal abdomen,' a case reported by Morgan.

I found the mutation 'bent wing' in a race with bifid wings and vermilion barred eyes, all three of these characters being in Chromosome I (sex-linked). A cross of bifid vermilion barred bent male by a pink-eyed female (pink is in Chromosome II) gave in F_1 all the males normal, and the females also normal except in respect to barred, which is dominant; in the next generation, F_2 , all combinations of the factors concerned appeared. If bent had been in Chromosome III, no crossing-over would have occurred between bent and pink in the F_1 male, consequently, no pink bent spermatozoa could have been formed, and thus (since pink and bent are both recessive) no pink bent F_2 individuals could have been produced. Since these were produced, bent did not lie in the *third* chromosome. Similarly, if bent had been in the first (X) chromosome or in the Y, no crossing-over between it and the sex-factor would have taken place in the P_1 or F_1 male, and consequently no bent females could have been produced in F_2 . As bent females were produced, bent did not lie in the *first* (X) chromosome, or in the Y.

A bent barred eyed F_2 male was then crossed to a female containing three mutant genes in the *second* chromosome, namely,

the genes for black body color, purple eyes, and curved wings. Here, too, some flies were obtained in F_2 which showed the characters of both grandparents at once (i.e., were both bent, and black, purple, and curved, and sometimes also barred). This proved that bent did not lie in Chromosome II. The details of the count are shown in table 1.

TABLE 1

Not bent

WILD TYPE	BLACK PURPLE CURVED	BLACK	PURPLE CURVED	CURVED	BLACK PURPLE
84	30	1	3	6	5

Bent

43	9	1	1		
----	---	---	---	--	--

The number of barred and non-barred, also of males and females, in each class of moderately large size, were approximately equal. The linkage manifested between black, purple, and curved corresponds as closely with expectation based on the previous linkage results of Bridges, as could be demanded for the small numbers involved. The determination of curved was at times uncertain, owing to the tendency of bent to curve too, and the determination of purple in eyes which were barred was also sometimes uncertain. But as far as the results go, they show that bent is independent of black, purple, curved, barred, and sex.

As no counts had been made in the cross with pink, and few were obtained in the cross with black purple curved, it was still conceivable that although bent was not absolutely linked, in the male, with the members of one of the three previously known groups, as has always been found to be the case with other genes, still it might perhaps be partially linked, in either or both sexes. New crosses were therefore made with the object of securing accurate counts. Some bent males descended from F_2 of the cross with pink were mated to black pink females. These males were found to be heterozygous for pink, as half of the F_1 flies were pink (although none were black or bent). The red-eyed F_1 flies were

mated together, to show the distribution of bent with respect to all three previously known chromosomes. The pinks were also mated together. The latter cross should show the distribution of bent with respect to Chromosomes I and II only, as all the flies were homozygous for pink, the mutant gene in Chromosome III.

The composition of the pink flies used as parents, with the paternal and maternal genes which they contained, was as follows:

F ₁ male	Paternal genes	--	Gray	Pink	Bent
	Maternal genes	sex	Black	Pink	Straight
F ₁ female	Paternal genes	sex	Gray	Pink	Bent
	Maternal genes	sex	Black	Pink	Straight

The count of offspring of this cross of pink flies resulted as shown in table 2.

TABLE 2
All individuals pink

		WILD TYPE	BLACK	BENT	BLACK BENT
F ₂ numbers observed	females...	83	30	24	8
	males....	77	24	31	9
	Total....	160	54	55	17
Total numbers expected on independence		162	54	54	18

Numbers of females expected = numbers of males expected.

The results conform with the theoretical expectation on the assumption of independent segregation between black, sex and bent.

The composition of the parents in the cross of red flies was as follows:

F ₁ male*	Gray	Red	Bent
sex	Black	Pink	Straight
F ₁ female	Gray	Red	Bent
sex	Black	Pink	Straight

* The paternally derived allelomorphs are on the upper line, the maternal on the lower.

The offspring were as seen in table 3.

TABLE 3

		WILD TYPE	BLACK	PINK	BENT	BLACK PINK	BLACK BENT	PINK BENT	BLACK PINK BENT
F ₂ numbers observed	females..	87	40	36	17	5	6	12	3
	males....	98	27	39	29	17	10	10	3
	Total....	185	67	75	46	22	16	22	6
Total numbers expected on independence		185	62	62	62	20.6	20.6	20.6	6.9

Numbers of females expected = numbers of males expected.

A considerable differential viability came into play in these bottles, as is proved by that fact that certain 'contrary' or opposite classes which were necessarily produced in equal numbers at fertilization (no matter whether linkage was involved or not), gave rather different counts of adult flies (e.g., 206 females; 233 males; 97 pink straight, 62 red bent; which were the worst discrepancies). On allowing for these differences obviously due to viability, we find that there are no discrepancies due to linkage of bent with black or pink, for the average between black straights and gray bents (pink plus red), is to the black bents as 3:1, and the average between pink straights and red bents (gray plus black) is to the pink bents as 3:1, as expected on independence. These results therefore confirm those previously obtained in regard to the independence of black (Chromosome II) and bent; they show in addition, however, that bent is independent of pink (Chromosome III).

The distribution of bent with regard to black and pink was next determined separately for each sex, by means of back-crosses. A normal male was mated to black pink bent females. The F₁ males, which were heterozygous for all three factors, as well as for sex, were then mated to triply recessive black pink bent females. If the factors were independent, all classes in the next generation should be equal in number. The actual result was as shown in table 4.

TABLE 4

CLASSES	WILD TYPE	BLACK	BENT	BLACK BENT	PINK	BLACK PINK	PINK BENT	BLACK PINK BENT
females	29	28	23	30	16	14	15	9
males	25	22	22	17	14	18	16	15
Total	54	50	45	47	30	32	31	24

Here practically the only irregularity not due to chance is obviously caused by a deficiency (low viability) of pink flies of all classes, and by a lower viability of males than of females. The independent assortment of bent with respect to the other factors, and of the other factors with respect to each other, is best brought out (especially in a case involving differential viability) by a tabulation of the percent of cases in which any two pairs of factors, considered by themselves, underwent recombination in the formation of the germ cells of the heterozygous parent. Thus, in the case of sex and bent; the factors for sex and for bent in the heterozygous male parent were both derived from the mother, the Y-chromosome and the normal allelomorph of bent ('straight') both coming from the father. Yet in the segregation division by which the sperm were formed, a recombination occurred as frequently as a persistence of the old combination, so that as many eggs were fertilized by sperm bearing the sex factor and straight, or the Y-chromosome and bent, as were fertilized by sperm with sex and bent or Y and straight. As a result, straight females and bent males formed 50 per cent of the total number, as we should expect on the basis that the factors were in non-homologous chromosomes which were assorted independently. The other per cents of recombination were as follows:

	per cent
Sex-black	50.0
sex-pink	55.0
black-pink	50.5
black-bent	50.5
pink-bent	49.0

In a reciprocal cross some of the triply heterozygous F_1 females were back-crossed to triply recessive males. Here, too, we should

expect all classes equal, barring differential viability, on the basis of independent assortment, and approximately 50 per cent of recombination between any two factors. The result was similar to that obtained in the back-cross of the males (table 5).

TABLE 5

CLASSES	WILD TYPE	BLACK	BENT	BLACK BENT	PINK	BLACK PINK	PINK BENT	BLACK PINK BENT
females.....	23	14	16	22	19	11	15	10
males.....	22	23	17	22	15	12	9	8
Total.....	45	37	33	44	34	23	24	18

The per cents of recombinations were as follows:

	<i>per cent</i>
Black-pink.....	54.0
black-bent.....	45.5
pink-bent.....	52.0

Here we cannot obtain the per cents of recombinations between sex and the other factors, since the parent which was heterozygous for the other factors was not the one which was heterozygous for sex.

CONCLUSIONS

The foregoing experiments prove that the gene for bent wings segregates independently of the sex-linked group of genes and of the two hitherto known non-sex-linked groups; accordingly, the genes of *Drosophila* now fall into four divisions, one sex-linked, corresponding to the X-chromosome, and three non-sex-linked, corresponding to the three pairs of autosomes. Thus the chief gap yet remaining in the series of genetic phenomena that form a parallel to the known cytological facts in *Drosophila ampelophila* has now been filled. It may therefore be predicted that no genes undergoing independent assortment of those at present known can hereafter be discovered in individuals of *Drosophila ampelophila* that show the chromosome configuration normal to the species, and it also seems probable that when other mutations are discovered in the fourth group, the genes in which they occur will be found to be linked strongly to the gene for bent wings, since

the fourth chromosome is probably the small one, and so any genes in it must lie near together. The close parallel existing between the number and relative sizes of the groups of genes and of the chromosomes leaves little doubt that it must be the pair of small chromosomes with which the factor for bent wings is connected, and that mutations occur more frequently in larger groups of genes, which are connected with larger chromosomes, than in smaller groups; mutation therefore would happen pretty much at random, in that it would usually take place about as often in one group as in another of equal size.⁵ This, too, makes it probable that the mutations in the larger chromosomes have occurred at various points scattered throughout their whole length, and are not confined, as a group, to a particular region or regions. The exceptional case of no mutations having been observed in the Y-chromosome, as we have seen, does not really form an argument against this view, which other facts support. That more mutations have been found in the X-chromosome than in either of the two long autosomes, which are nevertheless about the same size as X, is also to be expected, because a larger proportion of the mutations occurring in X would be noticed, since the male flies manifest all genes present in their single X, whereas in the case of other chromosomes, any mutant gene that is recessive to normal cannot manifest itself unless it be present in duplex. Granting, then, the correspondence between size and number of chromosomes and of groups of genes, it is difficult to see why larger groups of genes should follow the distribution of the larger chromosomes unless we conceive the connection between the genes and the chromosomes to be that the genes are material particles actually lying in and forming a part of the chromosomes with which they go. In any case, we must admit that the occurrence of a mutation in a fourth independent group of genes in *Drosophila* forms a further argument, if any more still be needed, in favor of the chromosome theory of heredity.

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⁵ This should not be taken to mean that any particular gene mutates as often as any other; it is definitely known that, both in *Drosophila* and in other forms (corn, *Marabalis*, etc.), some genes are more likely to mutate than others.

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THE EFFECTS OF CENTRIFUGING THE SPERMATOCYTE CELLS OF NOTONECTA, WITH SPECIAL REFERENCE TO THE MITOCHONDRIA

ETHEL NICHOLSON BROWNE

SIX FIGURES

The spermatocyte cells of *Notonecta* show a very definite distribution of substances, including the mitochondria, when subjected to a centrifugal force. The method of procedure was to dissect out the testes from the insects, put them together with a little Ringer's solution in small glass tubes and centrifuge them for two or three hours with a water centrifuge. The testes were then either teased out on a slide and the cells stained with an intra-vitam stain, Janus green, and examined, or were fixed in Flemming's fluid, sectioned and stained with iron hematoxylin.

The normal resting spermatocyte cells of *Notonecta* have been described in a former paper (Browne '13). The cytoplasm contains scattered mitochondria of two sorts, fibers and spheres, and the nucleus contains a deeply staining karyosphere in which the chromatin material is collected (fig. 1). When a cell of this sort is centrifuged, the mitochondria are driven to the distal¹ pole, where they appear as a deeply staining mass in which individual bodies cannot be distinguished except along the edge (fig. 2). The mitochondria are therefore the heaviest material in the cell. The rest of the cell is filled with clear cytoplasm. The nucleus goes to the centripetal¹ pole, showing that it is of less specific gravity than the cytoplasm. The karyosphere is always driven to the distal end of the nucleus, and in some cases in fact, is driven through the nuclear membrane.

¹ The term 'distal' is used in this paper to denote the pole away from the axis of the centrifuge, the term 'centripetal' to denote the pole nearest the axis of the centrifuge.

In younger spermatocyte cells, as I have described, there is besides the scattered mitochondria, a flat plate of mitochondrial material lying just outside the nuclear membrane, and a mass of mitochondria projecting from it into the cytoplasm. When cells of this type are centrifuged, this plate and mass of mitochondria remain in position, the rest of the mitochondria are driven to the distal pole (fig. 3). In these young cells, there is an aggregation of oil drops at the extreme centripetal pole; these can be distinguished only in the living material, probably being dissolved in the process of fixing and staining. The nature of these drops has been determined by means of Soudan III. The oil drops are not present in the older spermatocyte cells, having apparently been used up during the growth of the cells.

In the dividing cells, the mitochondria are likewise thrown to the distal pole by the centrifuge. The spindles and asters are, in many cases at least, perfectly normal and are not interfered with by the shifting of mitochondria, but lie in approximately the normal position in the clear protoplasm. Since it has not been determined how long one of these cells normally takes to divide, it is not possible to say whether the chromosomes actually pass to the poles while the cells are in the centrifuge, but various anaphase stages have been found in the centrifuged cells showing that the chromosomes are undergoing normal division. The spindle axis may lie in any direction with reference to the mitochondrial mass. In figure 4, the spindle axis is approximately parallel to the mitochondrial mass; in figure 5, approximately perpendicular. Figure 6 shows a spindle in polar view and its relation to the mass of mitochondria. In some cases, a few of the mitochondria apparently get caught by the spindle or asters and thus appear on the centripetal side of the division figure (figs. 5 and 6).

When the cells are centrifuged and then left in Ringer's solution for several hours or even overnight, the materials do not become redistributed over the cell, but they remain in the position induced by the centrifuge.

It is of interest to compare the centrifuged spermatocyte cells with centrifuged egg cells. In a number of marine eggs, it has

been found by Lyon ('07), Morgan ('10) and Lillie ('08) that there are at least three layers of materials segregated out by the centrifuge. There is at the centripetal pole a gray cap, then a layer of clear protoplasm, and at the distal pole a mass of yolk and pigment. This is true in the eggs of *Arbacia*, *Asterias*, *Nereis*, *Chaetopterus*, *Phascolosoma*, *Cerebratulus* and *Cumingia*. The gray cap, in some cases at least (*Nereis*, *Chaetopterus*, *Cumingia*) is formed of oil drops and corresponds therefore, in nature and in position to the cap of oil drops in the centrifuged spermatocyte cells. The middle layer of clear protoplasm is practically the same in the two cases. The heaviest material in the egg cells is the yolk and pigment, and this therefore corresponds with the mitochondria in the spermatocyte cells, but whether any of the mitochondria have the same function as the yolk spheres of the marine egg is questionable. In a very recent paper, Beckwith ('14) has shown that there are also three layers in the centrifuged eggs of *Hydraetia*, and she has been able to distinguish between yolk and mitochondria, which are mingled together at the distal pole. This is the first attempt, so far as I know, to segregate the mitochondria from the protoplasm by experimental methods, and it is interesting to find that her results for the egg tally with my own for the spermatocyte cells.

In comparing the unfertilised marine egg and the resting spermatocyte cell of *Notonecta* the resemblance is very striking. The normal cells in the two cases show a close similarity in appearance, and react in the same way to the centrifuge. In the case of the egg, the germinal vesicle is forced toward the centripetal pole by the centrifuge, and the nucleolus to the distal end of the nucleus. The nucleus and the karyosphere in the spermatocyte cells take the same relative positions. In the dividing cells of the marine eggs, it has been found in general that the mitotic figure remains intact when the cells are centrifuged, just as it does in the dividing spermatocyte cells. The whole figure may be shifted in the eggs so as to lie in the clear protoplasm. This does not occur to any considerable extent in the spermatocyte cells, though there might be a very slight shifting in some cases.

The main conclusions that can be drawn from these experiments are: (1) Three layers of materials can be segregated in the spermatocyte cells of *Notonecta* by means of the centrifuge, a cap of oil drops, a clear protoplasmic layer and a layer of mitochondria. (2) The mitochondria are definite bodies which can be separated from the protoplasm proper, and they are the heaviest material in the cells. (3) Normal division figures occur in spite of the redistribution of materials.

Marine Biological Laboratory,
Woods Hole, Mass.
July 7, 1914.

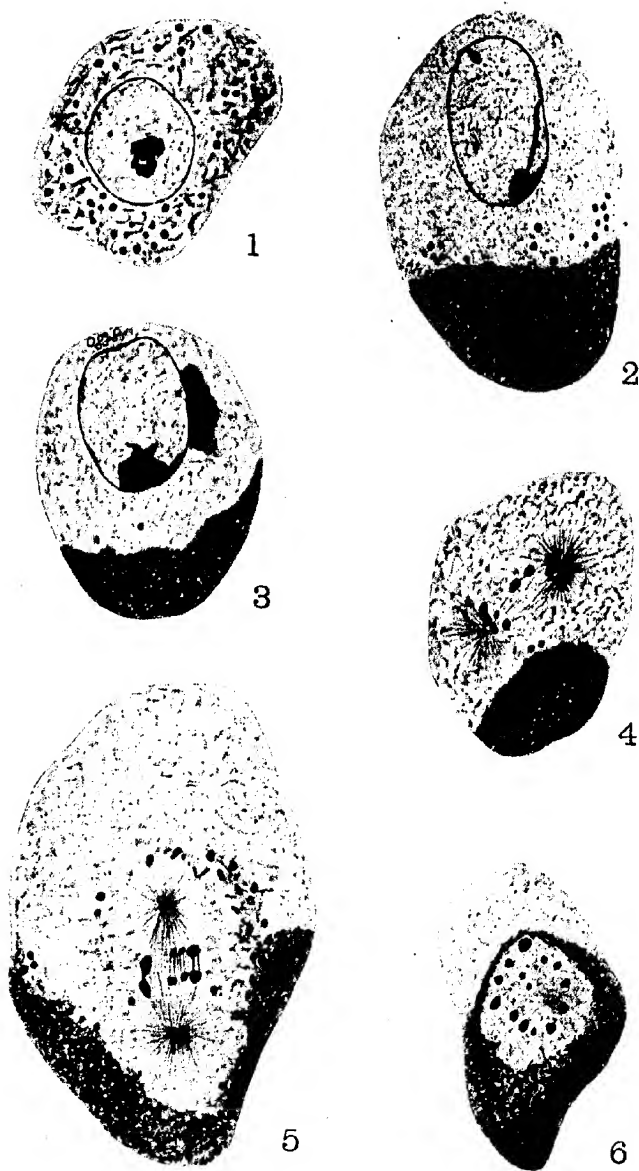
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PLATE I

EXPLANATION OF FIGURES

- 1 Normal resting spermatocyte cell, showing distribution of mitochondria.
- 2 Older resting spermatocyte cell, centrifuged. The gray mass consists of mitochondria.
- 3 Younger resting spermatocyte cell, centrifuged, showing oil drops, and mass of mitochondria against nuclear membrane.
- 4 Dividing cell, centrifuged; late anaphase.
- 5 Dividing cell, centrifuged; early anaphase.
- 6 Dividing cell, centrifuged; polar view of metaphase.



FERTILITY AND STERILITY IN DROSOPHILA AMPELOPHILA

III. EFFECTS OF CROSSING ON FERTILITY IN DROSOPHILA¹

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ONE DIAGRAM

CONTENTS

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¹ This part of the work was carried out at the Marine Biological Laboratory at Woods Hole, Mass. The writer is indebted not only to the management of the Station, but also to the Zoological Department of Columbia University for placing at his disposal every opportunity for carrying forward the present study. I wish to express my appreciation to Prof. T. H. Morgan for the unfailing interest he has taken in these studies.

INTRODUCTION

This paper, which is the third of the series on "Fertility and Sterility in *Drosophila ampelophila*," considers the evidence that bears on the effects of crossing on fertility when: (1) Stocks from the same source (descended from the same grandparents) separately inbred are crossed; (2) Stocks from different sources separately inbred are crossed; (3) When the mutations, having pink eyes and white eyes are crossed with each other and with a wild stock which has been inbred (brothers and sisters) for 26 generations.

The evidence so far presented goes to show that some of these stocks have, up to the time the present experiments were carried out, lost in fertility on inbreeding. The evidence, however, does not show conclusively that the loss is due to the process of inbreeding as such. The fact remains, that the different strains did up to this time lose in fertility on continuous inbreeding, and it will now be shown what effect out-crossing has on these reduced strains. It is to be recalled that 'fertility' is here used in the sense of eggs that reach maturity.

There are some experimental data that bear out the belief that the crossing of different races or strains is beneficial to the *fertility of the stock itself*. This relation has been demonstrated beyond any doubt in the second part of this paper in so far as it applies to the truncate mutant for the fertility of the female was more than doubled when mated to a wild stock, while the fertilizing powers of the truncate male were more than three times as great with a wild female as compared to his own mate.

I wish now to consider the evidence that bears on the question as to whether or not a rise in fertility will result when one strain of *Drosophila* is crossed with any other strain. The evidence deals especially with the fertility of two mutations and two wild stocks that originally came from two different sources. It will facilitate treatment of this subject to give a brief résumé of the history of these stocks.

HISTORY OF THE STOCKS USED IN CROSSING

1. *The Woods Hole stock*

This is a wild stock that originally came from Woods Hole, Massachusetts. How long it had been in captivity I do not know, but when I took charge of the stock it showed a fertility of 77.3 per cent. The stock was tested at various times throughout the year 1912-1913. The behavior of the fertility of this stock is given in table 1. The stock was cultured *en masse*.

TABLE 1
Showing the fertility of the Woods Hole stock

TIME TESTED	NO. EGGS ISOLATED	NO. FLIES HATCHED	PER CENT FERTILITY
September 11 to September —, 1912.....	1402	1084	77.3
September 16 to October —, 1912.....	1622	1227	75.0
January 20 to February 14, 1913.....	2085	1421	70.0
June 28 to July 15, 1913.....	917	581	63.4
July 15 to July 28, 1913.....	531	317	59.7
August 7 to August 24, 1913.....	601	431	71.7
August 17 to September 4, 1913.....	856	715	83.6
October 21 to November 11, 1913.....	467	341	73.0

2. *The inbred stock*

This stock originally came from Falmouth, Massachusetts. During the course of the experiments brothers and sisters have been selected and paired for over 26 generations. Its productivity was at the beginning of the experiments relatively high as shown by the large number of offspring produced. On inbreeding the stock gradually dropped in productivity as shown in table 2.

TABLE 2
Showing the productivity of the inbred stock in successive generations of close inbreeding

GENERATION	1	2	3	4	5	6	7	8	9	10	11	12	13	14
No. offspring produced....		368	209	190	184	65	119							159

This evidence taken by itself does not prove conclusively that the loss in productivity was due to a decrease in the fertilizing power of the gametes. That this is a very probable interpretation however is shown by the fact that in the F_{11} generation when the productivity dropped to 159 per pair their fertility was only 32 per cent. And yet the flies of the F_{11} generation were producing twice as many fertile sperm and twice as many fertile eggs as shown by outcrossing into the truncate stock (Part II, diagram E).

3. I_2 and I_3 stocks

In the fifth generation of the inbred stock, when they were producing 184 offspring to the fertile pair, I set aside two different bottles besides a number of pairs that were kept to continue the inbred stock. All of these flies had descended from the same grandparents, a single pair of the third generation. In one bottle there were three females and four males. This I shall designate as I_2 . In the other bottle there were ten males and twenty-two females. This I shall refer to as I_3 . These flies were set apart April 2, 1912, and received no attention except that the flies were transferred to clean bottles about every three weeks and fresh food added from time to time. It is important to bear in mind that we have three stocks descended from the same germplasm.

4. The white-eyed stock

This stock arose as a mutation from the inbred stock early in the history of the strain. This stock received the same treatment as I_2 and I_3 .

5. The pink-eyed stock

This is an eye mutation that arose in one of Morgan's cultures.² It had been bred for some three years. I received my stock from Mr. Liff, a graduate student in the department, who has made a study of their productivity. This stock was bred in mass culture for about four months when the present experiments were carried out.

² Science, vol. 33.

We have then six different strains, two from different localities and some that had the same germ plasm. I now propose to examine the fertility of the different stocks and their behavior on crossing. The combinations were made up as shown in diagram A.

METHODS

In order to get an exact measure of fertility the eggs were isolated as described in Part II. From the stock bottle in each case 15 virgin females and 30 young males were selected. The flies that hatched from June 19 to June 26 were separated every twelve hours from the stock bottles. There can be no question as to the virginity of the females. The males and females were kept in separate bottles and the different combinations made up on June 26. An epidemic of mating took place in all the bottles a short time after the flies came out from under the influence of the ether.³ I commenced to isolate the eggs two days later. This process was carried out in the same way as in previous experiments with one exception. The weather was very warm and the larvae emerged from the egg in less than twenty-four hours. In order to exclude this source of error I added another bit of food after isolating the eggs. This served as food for the parents. After six or eight hours this food was removed and a new bit added from which the eggs were later isolated. Tables 3a, 3b, and 3c give the number of eggs isolated each day and the corresponding number that hatched.

DISCUSSION

A study of diagram A brings out the essential relations that concern the questions propounded at the beginning of this paper. 1. What is the effect on fertility when germ plasm originally from the same source separately inbred for several generations is recombined by crossing? It will be recalled that I, I₂ and I₃ represent in this case the stocks under study. It is to be noted that the three stocks although originally brothers and sisters and descended from

³ Mr. A. H. Sturtevant has shown that the mating habit in this species is largely associated with the sense of smell and this fact probably accounts for the phenomenon here observed.

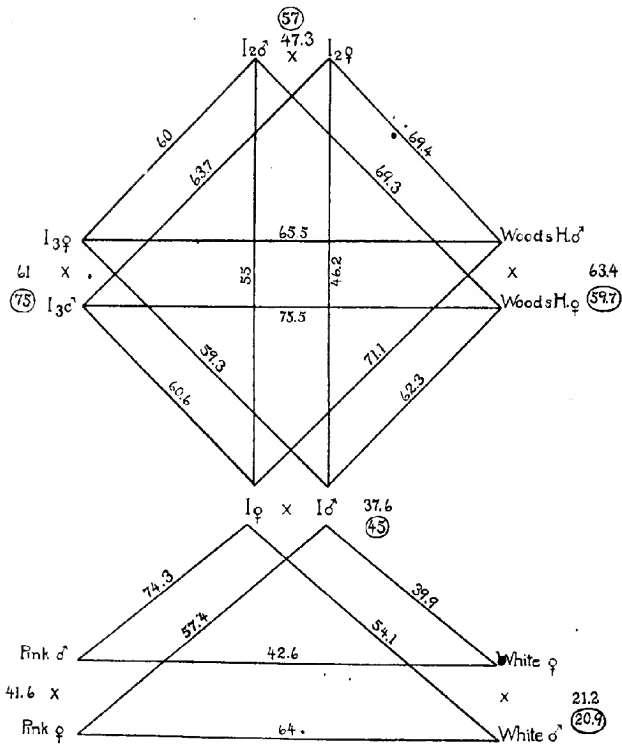


Diagram A Based on the foregoing data, showing different combinations and the fertility in each case.

the same grandparents, show different degrees of fertility. The inbred stock in which brothers and sisters had been continually selected had lost most in fertility. In the crosses it will be observed that there is no sudden rise in fertility as one might expect but, on the other hand, a peculiar relation exists in that the stock in each case having the highest fertility is able on crossing to bring the fertility of the lower stock up to its level and this is true whether through the male or through the female.

TABLE 3 a

Showing the number of eggs isolated day by day and the corresponding number that hatched from each of the combinations. The first letter in each combination represents the stock from which the female was taken, the second letter the stock from which the male was taken. T = truncate; I = inbred, I_1 = second inbred stock from 3 males, 4 females; I_2 = third inbred stock from 10 males, 22 females, WH = Woods Hole stock; P = pink-eye stock; W = white-eye stock

DATE 1913	1 (control) $T \times T$		2 $I_1 \times I_2$		3 $I_2 \times I_1$		4 (control) $I_1 \times I_1$		5 $I_2 \times I_2$		6 (control) $I_1 \times I_2$		7 $I_2 \times WH$	
	Eggs	Adults	Eggs	Adults	Eggs	Adults	Eggs	Adults	Eggs	Adults	Eggs	Adults	Eggs	Adults
June 28.....	0	0	48	27	37	21	80	39	60	25	40	23	25	21
29.....	36	7	21	17	34	24	35	24	47	27	61	29	56	42
30.....	56	14	50	32	29	20	55	32	60	31	36	16	0	0
July 1.....	50	13	30	19	40	26	40	29	48	25	60	26	38	31
2.....	37	4	25	12	40	17	25	7	32	21	71	18	20	6
3.....	48	2	40	18	33	20	33	14	0	0	49	25	22	9
4.....	45	7	30	14	65	26	22	11	60	26	50	23	26	13
5.....	52	6	56	27	57	39	73	41	52	21	85	20	50	33
6.....	42	10	64	36	36	16	50	14	26	7	75	31	50	34
7.....	50	11	75	51	50	29	67	55	75	61	55	35	55	41
8.....	20	9	33	23	28	24	17	15	36	20	24	10	12	10
9.....	22	5	28	21	24	12	30	25	42	26	44	17	0	0
10.....	43	4	75	30	60	26	35	24	50	26	75	40	25	12
11.....	60	13	40	24	0	0	50	35	60	25	60	37	60	46
12.....	30	8	50	39	75	60	21	19	75	44	75	38	37	24
13.....	9	5	25	16	40	22	20	16	55	34	65	41	13	6
14.....	0	0	30	26	33	25	0	0	35	30	40	26	17	4
15.....	0	0	20	11	20	9	0	0	15	8	20	11	16	7
Total.....	600	118	740	443	701	416	653	400	828	457	985	466	522	339
Per cent.....	20		60		59.3		61		55		47.3		61.5	

TABLE 3b

8 (control) WH X WH		9 I ₂ X WH		10 I X WH		11 I X I ₂		12 I ₂ X I ₂		13 (control) I X I		14 I ₂ X I		15 WH X I ₂		16 WH X I	
Eggs	Adults	Eggs	Adults	Eggs	Adults	Eggs	Adults	Eggs	Adults	Eggs	Adults	Eggs	Adults	Eggs	Adults	Eggs	Adults
36	20	47	30	35	25	22	11	25	11	10	4	38	20	53	46	35	21
18	11	31	20	60	45	24	20	21	11	18	13	42	21	42	30	65	33
40	24	27	16	50	26	45	25	38	28	71	37	25	12	62	57	62	51
28	19	35	22	45	23	42	22	0	0	26	8	60	33	48	38	40	36
18	7	20	7	25	13	30	16	35	25	30	13	40	24	50	27	19	13
15	10	61	42	45	29	28	13	41	19	17	6	29	13	100	50	31	20
55	28	8	4	25	19	35	17	50	26	31	7	45	15	50	30	65	23
51	28	43	25	63	53	68	27	90	37	60	15	64	27	100	63	35	15
80	47	50	29	46	28	30	18	67	30	40	10	75	31	52	50	40	13
80	60	41	33	75	65	80	57	66	45	40	18	50	31	100	80	90	67
100	69	23	20	32	21	30	21	12	9	45	18	38	21	64	56	45	38
60	46	35	32	34	38	30	16	31	22	22	6	23	8	57	47	40	33
76	38	0	0	64	34	50	26	35	20	50	17	65	25	72	45	75	32
60	30	60	44	60	44	40	22	40	25	50	14	60	20	60	30	60	20
55	35	70	61	100	84	80	64	75	63	60	25	50	35	68	64	70	49
63	53	40	29	40	26	20	11	60	50	43	17	39	24	75	70	55	40
42	32	45	33	40	35	35	26	34	21	15	11	30	12	40	39	50	44
40	24	30	15	40	29	40	30	20	9	20	7	30	0	40	33	30	17
917	581	666	462	896	637	729	442	740	471	657	246	805	372	1133	855	907	565
63.4		69.4		71.1		60.6		63.7		37.6		46.2		75.5		62.3	

TABLE 3c

TABLE 3c																	
17 WHX I ₂		18 W X I		19 I X P		20 P X I		21 (control) P X P		22 W X P		23 (control) W X W		24 I X W		25 P X W	
																Eggs	Adults
Eggs	Adults	Eggs	Adults	Eggs	Adults	Eggs	Adults	Eggs	Adults	Eggs	Adults	Eggs	Adults	Eggs	Adults	Eggs	Adults
45	30	41	22	0	0	10	0	36	22	50	38	28	11	10	5	60	46
53	44	40	18	0	0	0	0	30	9	16	4	20	4	14	10	38	26
60	48	18	9	32?	11	10	5	35	23	15	5	33	8	31	23	80	63
50	33	36	14	20	14	21	17	32	17	26	16	25	10	28	13	61	38
35	21	22	8	14	10	26	16	34	7	24	4	7	4	26	17	42	15
40?	26	31	11	7	5	20	10	56	15	7	2	42	8	22	13	47	24
82	42	55	15	10	3	55	28	20	4	43	12	35	1	17	2	30	7
58	38	20	7	47	31	80	53	75	28	14	2	40	3	85	50	85	38
48	31	35	6	19	16	18	6	50	15	17	8	10	0	21	9	41	19
90	76	27	9	80	60	40	23	40	19	40	24	50	9	23	11	60	47
75	66	8	0	37	34	0	0	7	5	10	5	40	7	34	16	31	21
54	41	15	3	26	19	17	13	24	17	13	7	50	13	27	11	50	43
65	30	21	7	60	44	43	20	46	10	35	8	29	2	60	31	81	50
50	10	00	20	60	46	70	31	60	17	50	21	65	13	70	30	70	35
65	59	50	32	70	60	85	54	80	44	45	23	55	19	60	41	40	40
40	41	43	25	30	24	60	20	20	12	22	42	10	65	12	45	17	39
30	28	33	21	40	36	40	30	50	24	42	18	35	7	27	19	26	17
20	14	35	7	20	12	20	12	40	18	40	10	30	7	10	4	18	15
979	678	580	234	572	425	615	353	735	306	506	217	640	138	613	332	910	583
69.3		30.7		74.3			57.4	41.6		42.6		21.2		54.1		64	

The second question under consideration is; What is the effect on fertility when stocks from different sources separately inbred are crossed? The relations expressed by the right hand side of diagram A throw some light upon this question. In two of the combinations high fertility brought the low fertility up to its level while in four of the combinations there is an appreciable rise in fertility beyond that shown by the parents, although by no means as high as one might expect from the history of the crosses made with the truncates in Part II. But since the rise in fertility occurs here in the combinations expressed by the right side of the diagram and not those on the left it looks as if the rise in fertility in this case is significant. It must be admitted, however, that the rise in fertility in these crosses is not great enough to base a final conclusion upon, in regard to a point as far-reaching as this. It is to be remembered that Woods Hole, Massachusetts, is only four miles distant from Falmouth and it is altogether probable that the two strains had not been separated by many generations when taken into captivity. Consequently, this material would present after all a picture very much like the first case. If the environment can influence the different strains in respect to the 'factors' that bring about fertility the influence in this case has been slight, and, after all, from stocks so closely related this is probably what we should expect to find. In any case it is certain that the high-producing stocks can bring the fertility of the low-producing stocks to their level whether descended from the same or from different germ plasms. It might seem from this (although I am far from contending at present that such is necessarily the case) that there is a set of factors of some sort for fertility and that when a loss in fertility occurs in a stock (other than that which occurs in the case of mutations) it is the same set of factors that is lost or is changed and this accounts for the fact that there is no rise in fertility on crossing. The stock that has the highest fertility (the largest number of factors) acts as a dominant character and brings the lower set of factors up to its level. In the case of a sudden rise in fertility on crossing such as occurs in the case of some of the mutations it is probable that the stocks have lost different factors for

fertility, and that crossing gives the proper constellation of factors for a marked rise in fertility.

It must be frankly admitted that this explanation at best is only tentative and that the door must be left open for further investigation. The possibility of transmissible lethal factors is not to be overlooked.

The third question under consideration relates to the effect on fertility of crossing certain mutations, namely, pink-eye and white-eye and the effect on fertility when these are crossed into the inbred stock. The triangular portion of diagram A shows that there is a rise in fertility in all cases. In four of the six cases the rise in fertility is very high. This is analogous to what happens in the case of the truncates when crossed into other stocks.

I wish here to add the data from an experiment that has some bearing on the foregoing considerations and also some bearing on the question of inbreeding. The object of this experiment is to serve as a check upon the controls used in the foregoing experiments, namely, Woods Hole, white-eye, I_2 , I_3 and the inbred stocks. This experiment was carried out in the same manner as the foregoing. The egg counts began the same day that the former experiment closed, July 15, 1913. The number of eggs isolated, together with the corresponding number that hatched, is given in table 4.

I have placed the percentage of fertility of these stocks in diagram A and enclosed the numbers in circles. It will be noted that the Woods Hole and white-eyed stocks remain practically the same as in the previous experiment. There is a marked rise in fertility, however, in case of the three stocks originally from the same germ plasm. Later (Aug. 7 to 24, table 1) the Woods Hole stock was tested. Its fertility had risen from 59.7 to 71.7. The Woods Hole stock was again tested August 17 to September 4. This stock now gave a fertility of 83.6. The fact is that all my stocks at this time showed a marked rise in fertility, as is evidenced by the experiments to be dealt with in the next paper. Even the truncate stock which had been tested many times through the year and had varied from 20 to 26 per cent now gave a fertility

of 31 per cent. Table 1 shows that in the case of the Woods Hole stock the fertility gradually fell throughout the year. I fully expected to find that the fertility would be continually reduced on inbreeding, and yet the fertility of the stock rose in August and September until it was actually higher than it was during the September of the previous year by 6.3 per cent. The meaning of this is obscure. It will be recalled that Castle found seasonal fluctuations in productivity in his flies. The period, however, in which low productiveness prevailed in his stocks corresponds in a general way with the period of high fertility in my stocks.

In the light of this evidence it would seem that inbreeding as such cannot be the *vera causa* of the low fertility that usually accompanies the process. It would seem from this evidence and the evidence presented by Moenkhaus that the fertility of a stock could be maintained, and the closest inbreeding practiced, provided

TABLE 4

Showing the fertility of the Woods Hole, white-eyed, inbred, I_1 and I_2 stocks. 1A = Woods Hole stock; 2A = white-eyed stock; 3A = I_1 stock; 4A = I_2 stock; 5A = inbred stock, F_{24} generation

DATE 1913	1A		2A		3A		4A		5A	
	Eggs	Adults	Eggs	Adults	Eggs	Adults	Eggs	Adults	Eggs	Adults
July 15	95	65	75	21	70	58	53	33	75	32
16	50	34	33	4	40	31	35	21	67	31
17	45	30	50	15	40	35	50	40	30	14
18	44	31	30	8	50	42	60	41	32	15
19	29	14	51	20	45	34	50	26	50	26
20	30	10	50	11	66	40	45	22	30	11
21	31	15	31	2	40	33	25	7	35	16
22	41	24	25	3	35	30	35	14	12	6
23	21	11	31	4	25	19	40	22	20	6
24	30	21	26	3	17	13	40	26	16	9
25	25	12	25	3	17	6	22	8	22	12
26	50	29	25	5	32	27	22	16	33	18
27	25	13	11	2	37	26	25	14	20	10
28	15	8	35	5	30	18	25	12	25	6
Total.....	551	317	498	106	544	412	527	302	467	212
Per cent.....	59.7		20.9		75.7		57.3		45.4	

the proper combinations were made. It seems probable in the case of fertility, as in many other characters, that inbreeding gives a chance for defects to be brought to the surface; and that low fertility is likely to accompany close inbreeding provided it is not guarded by rigorous selection. When a stock has reached a low degree of fertility it seems strange that that same stock should be able to rise again in fertility. Yet this is exactly what may happen. Take for example the truncate fly which has been selected for 75 generations and has its fertility reduced to about 20 per cent; and yet that fly can throw a form, the long wings, the fertility of which is more than twice as great as its truncate brothers and sisters.

The fact that different individuals, brothers and sisters of the same stock, should differ in such a marked degree (so that one is actually able to separate the more fertile ones from the less fertile flies by inspection) is submitted as evidence to show how selection may operate in controlling the fertility in these strains.

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FERTILITY AND STERILITY IN *DROSOPHILA* *AMPELOPHILA*

IV. EFFECTS ON FERTILITY OF CROSSING WITHIN AND WITHOUT AN INCONSTANT STOCK OF *DROSOPHILA*

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ELEVEN DIAGRAMS

INTRODUCTION

In Part II of these studies it was shown that although the mutant stock truncate, produced a large number of fertile sperm and fertile eggs, yet when the truncate female was mated to the truncate male only 20 per cent of the eggs hatched. It was also shown that the truncates were not a homogeneous stock, for the flies with truncate wings give rise to offspring some of which have long wings like those of the wild flies. This has held true through many generations of continuous selection. These long winged flies in turn are also not homogeneous because they throw some truncates, and this has held despite some twenty (estimated) generations of selection.¹ A peculiar phenomenon shown by the long wings is that their fertility when tested together is about twice as great as that shown by their truncate brothers and sisters when tested together.

I wish here to present in detail the evidence that bears on the result of crossing within this inconstant stock; and the effects on fertility when both forms are crossed into a wild stock—the Woods

¹ Since both forms are under study in this paper it is convenient to refer to this stock as an "inconstant stock."

Hole stock. I shall also consider the behavior of the fertility of the extracted truncates (extracted after crossing out to a wild stock) when tested together and when back-crossed into their low-producing and high-producing grandparents.

FERTILITY OF THE EXTRACTED TRUNCATES

I wish to deal first with the evidence that bears on the question raised in Part II, as to whether or not the fertility of the truncate stock can be raised by outcrossing. In other words is low fertility in this case a concomitant of the truncate wing condition, or can high fertility be transferred to the truncate stock by crossing it out to a wild stock and extracting?

To answer this question a truncate female was crossed to a Woods Hole male. This cross I shall refer to as A. The reciprocal cross in which the truncate male was mated to the Woods Hole female I shall refer to as B. There were five bottles of the P_1 made up in each case. A large number of the F_1 were mated in pairs and the truncates selected from their offspring.

The fertility of the extracted truncates was then tested by means of the following combinations:

1. A truncate female (F_2 extracted from the truncate grandmother) was mated with four of her truncate brothers.
2. A truncate female (F_2 extracted from the truncate grandfather) was mated with four of her truncate brothers.
3. Control: The fertility of the original truncates was tested by placing a number of males with a single female in each case.

The results of this test are recorded in tables 1, 2 and 3. The fertility of the extracted truncates when tested together is almost 50 per cent, while the fertility of the truncates used for control is only 22.6 per cent. In other words, from a source of fertility of 63.4 per cent (Woods Hole stock; Part III, table 3b, no. 8) there has been transferred to the truncate winged forms (originally with a fertility of about 20 per cent) about 25 per cent of additional fertility. The increase in fertility is the same in this case whether the truncate male or female is used as the grandparent.

TABLE 1

Showing the result of testing the fertility of the A ♀ × A ♂ (F₂ truncates extracted from truncate grandmother)

NO.	TOTAL NO. EGGS LAID	NO. EGGS ISOLATED	NO. FLIES EMERGED	AGE OF ♀ WHEN COUNT BEGAN	LIFE OF ♀ IN DAYS	AVERAGE NO. EGGS LAID PER DAY	PER CENT OF EGGS WHICH COMPLETE DEVELOP- MENT
11	861	353	128	5	47	25.0	33.4
12	211	184	80	5	30	8.5	43.4
13	17	17	2	5	18		11.8
15	513	328	180	5	31	19.8	54.8
16	575	370	182	5	35	19.2	49.2
17	403	250	118	5	33	14.4	47.2
24	356	292	134	4	17	27.4	45.8
25	610	392	210	4	32	21.8	53.5
26	445	310	176	4	25	21.2	56.7
27	552	370	198	4	29	22.1	53.6
28	413	331	168	4	24	20.7	50.8
29	791	441	210	4	41	21.4	47.6
Total.....		3638	1786				

Per cent of eggs which complete development 49.1.

TABLE 2

Showing the result of testing the fertility of the B ♀ × B ♂ (F₂ truncates extracted from truncate grandfather)

NO.	TOTAL NO. EGGS LAID	NO. EGGS ISOLATED	NO. FLIES EMERGED	AGE OF ♀ WHEN COUNT BEGAN	LIFE OF ♀ IN DAYS	AVERAGE NO. EGGS PER DAY	PER CENT OF EGGS WHICH COMPLETE DEVELOP- MENT
16	581	370	182	5	35	19.4	49.2
20	*	1	1	5	25		
21	207	231	78	5	27	13.5	33.7
22	352	279	158	5	25	17.6	56.6
23	67	42	13	5	22	4.0	31.0
30	657	463	242	4	29	26.3	52.3
32	266	266	116	2	23	12.6	43.6
Total.....		1652	790				

Per cent of eggs which complete development 47.8.

*Laid eggs which went to pieces.

TABLE 3

Control: Showing the result of testing the grandparental stock, truncate by truncate

NO.	TOTAL NO. EGGS LAID	NO. EGGS ISOLATED	NO. FLIES EMERGED	AGE OF ♀ WHEN COUNT BEGAN	LIFE OF ♀	AVERAGE NO. EGGS PER DAY	PER CENT OF EGGS WHICH COMPLETE DEVELOP- MENT
1	154	26	4	7	28	7.3	15.4
3	62	62	16	7	16	7.0	25.8
4	287	265	73	7	26	15.1	27.5
5	227	227	43	7	35	8.1	18.9
7	36	36	10	6	14	4.0	27.8
8	258	238	54	6	27	12.3	22.7
10	226	126	22	6	41	6.4	17.5
Total		980	222				

Per cent of eggs which complete development 22.6.

It is true that the extracted truncates vary somewhat in the length of wing. Moreover, they appear in about the ratio of 1 truncate wing to 14 of the long wings. In all cases I selected the most typically truncate forms, and if low fertility is an accompaniment of the truncate wing as such, I should have discovered the fact in this experiment. This does not exclude the possibility, however, that some of the low fertility here may be concomitant to the truncate wing, for a comparison of the fertility of their long winged brothers and sisters shows that the fertility of the extracted longs is higher (see table 7).

In Part II it has been demonstrated by numerous experiments that the truncate stock is deficient in egg production. A comparison of the egg production of the extracted truncates together with that of the truncates used in control and also with that of the truncates given in Part II makes it absolutely certain that these extracted truncates have been benefited also in regard to egg productivity as a result of crossing and extracting from a wild stock. The evidence is conclusive that fertility can be transferred to the truncates.

PRODUCTIVITY OF THE EXTRACTED TRUNCATES

If it be true that the fertility of the extracted truncates is more than twice as great as the original truncates and if it be true that the egg production of the extracted truncates is increased, it follows that if the extracted truncates be bred together in pairs their productivity should be greatly increased. To judge from the previous history of the truncates in regard to productivity (Part II; table 1) and the attendant low fertility we should expect that by doubling the fertility of the gametes of the truncates that they would produce between 100 and 125 offspring on an average to the fertile pair instead of 50 as formerly. The number of offspring produced above 125 would, in a rough way, give a measure of the increased egg production. This does not take into consideration, any difference that may exist in the length of life between the truncates and extracted truncates. It would seem from the tables that the extracted truncates had been benefited to some slight degree in respect to the length of life as a result of extracting from a wild stock.

In order to throw some light on the question just raised, I bred together a number of these extracted truncates in pairs. These flies were from the same source as those used in the previous experiments. The pairs were made up August 9 and 10 and discontinued on September 1. The final count was made September 11; consequently the total number of children produced is not represented. The results are given in tables 4a and 4b. Ten pairs of truncates were used to control this experiment but it was evident from inspection that their productivity was practically the same as in former experiments and consequently the offspring were not counted. A study of the table makes it certain that it is possible not only to put fertility into the truncate stock but also increased egg production. Consequently the productivity of the truncates has been raised from 50 to about 300 as a result of crossing out and extracting. Since the fertility of the extracted truncates is twice as great and the productivity six times as great it follows that egg production has been increased three fold—from 200 to

about 600. A comparison of tables 1 and 2 where the egg production of the extracted truncates is given with table 3 and the many tables in Part II where the total output of eggs of the original truncates is represented will show that the above conclusion is warranted.

TABLE 4a

Showing the result of breeding together the extracted truncates. $A\varphi \times A\sigma$ (F_2 truncates extracted from truncate grandmother)

NO.	TOTAL NO. OFFSPRING
1	336
2	295
3	408
4	330
6	364
7	no offspring
8	299
14	250
15	271
16	265
17	211
18	228

TABLE 4b

$B\varphi \times B\sigma$ (F_2 truncates extracted from truncate grandfather)

NO.	TOTAL NO. OFFSPRING
5	203
9	196
10	390
11	162
12	363
13	127
19	no offspring
20	123

FERTILITY OF THE EXTRACTED TRUNCATES BACK-CROSSED TO THEIR GRANDPARENTS, THE ORIGINAL TRUNCATE STOCK AND THE WOODS HOLE STOCK

The foregoing pages have demonstrated the marked difference in fertility that exists between truncates and extracted truncates. I wish here to consider the effects on fertility when the extracted truncates are back-crossed to their low-producing grandparents (truncates) and their high-producing grandparents (Woods Hole). I shall consider first the evidence that bears on the effect on fertility of back-crossing extracted truncates with truncates. This experiment was carried out at the same time as experiment I in this paper. The facts are recorded in table 5.

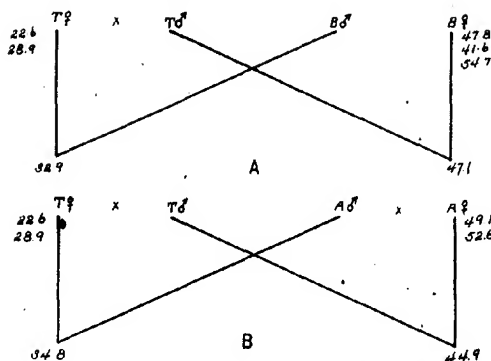


Diagram A Expresses the effect on fertility of crossing the extracted truncates B (in which the Truncate male was used as the grandfather) with the original truncates, T.

Diagram B Expresses the effect on fertility of crossing the extracted truncates A (in which the truncate female was used as the grandmother) with the original truncates, T.

The flies of table 5 were mated July 21 and the egg count began July 25. The sexes were kept separate for about four days before mating. The results of these crosses are brought together for comparison in diagrams A and B.

TABLE 5

Showing the effect on fertility of back-crossing the extracted truncates with the original truncate stock. T = truncate, all from family No. 178. A = truncate extracted from truncate grandmother. B = truncate extracted from truncate grandfather

NUMBER	COMBINATION	NO. OF FEMALES	NO. OF MALES	NO. EGGS LAYED	NO. FLIES EMERGED	PER CENT OF FERTILITY	TRUNCATES	LONGS
33	B ♀ × T ♂ No. 178.....	10	20	938	442	47.1	102	117
34	A ♀ × T ♂ No. 178.....	8	20	811	365	44.9	149	88
35	B ♂ × T ♀ No. 178.....	10	23	613	201	32.9	41	72
36	A ♂ × T ♀ No. 178.....	11	21	247	86	34.8	21	15
37	Control B ♀ × B ♂.....	7	15	637	265	41.6	64	105
38	Control A ♀ × A ♂.....	7	18	754	396	52.6	148	147
39	Control T ♀ No. 178 × T ♂ No. 178	15	34	668	194	28.9		
40	Control B ♀ × B ♂.....	12	21	931	510	54.7	80	120

In table 5 I have added as a matter of record the number of long-wings and short-wings that appeared in a number of bottles from which complete counts were made. No definite ratios can be given for comparison with the study of wing ratios made in Part II, where it was shown that the truncates threw 1 long to $7 \pm$ truncates, the longs in turn threw 1 truncate to $7 \pm$ longs. The ratio of the longs to the truncates in the crosses made here is near equality.

The foregoing experiments bear on the effects on fertility when the extracted truncates are back-crossed with their low-producing grandparents. I wish next to consider the effects on fertility when the extracted truncates are back-crossed with their high-producing grandparents—the Woods Hole stock. The experiment was carried out in the usual way. The results are given in table 6.

The relations brought out in this experiment are expressed in diagrams C, D, and E.

Diagrams A and B show the result on fertility of back-crossing the extracted truncates with the low-producing grandparental truncates. It is to be noted first of all that the controls show the fertility of the extracted truncates to be practically twice that of the original truncates. Moreover, the fertility of the extracted truncates is the same whether the truncate male or female is used as the grandparent. On crossing, there is no great rise in fertility beyond that shown by the parents, as has been demonstrated in the case of the truncates crossed to wild races. The extracted truncate female is able to bring the fertility of the truncate male up to its level but not beyond. The extracted truncate male, on the other hand, does not bring the fertility of the truncate female up to its level by about 15 per cent. It is to be noted that the fertility shown in diagram A is practically the same as in diagram B, indicating that here the effect is the same on the truncate grandchildren, whether descended from the truncate male or truncate female.

In contrast with the conditions found in these crosses, we find, that when the extracted truncates are back-crossed to the high-producing parents there is a sudden and distinct rise in fertility; beyond that of either parent stock (diagrams C and D). The

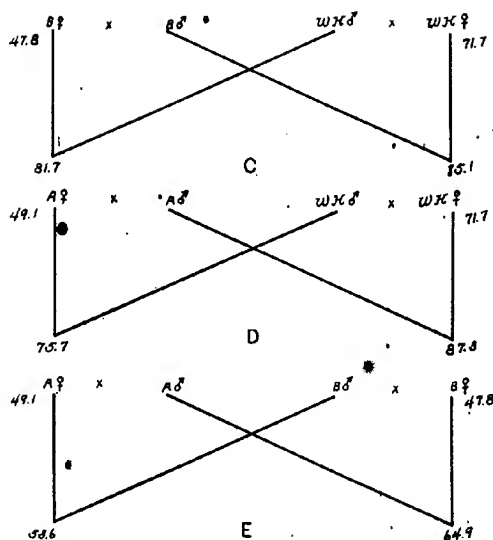


Diagram C Showing the effect on fertility of crossing the extracted truncates B (F_2 truncates descended from the truncate σ) with the high-producing grand-parental stock.

Diagram D Showing the effect on fertility of crossing the extracted truncates A (F_2 truncates descended from the truncate \circ) with the high-producing grand-parental stock.

Diagram E Showing the effects on fertility of crossing the extracted truncates A (F_2 truncates descended from the truncate \circ) with the extracted truncates B (F_2 truncates descended from truncate σ).

behavior of the fertility of the extracted truncates toward the Woods Hole stock is practically the same whether the truncate flies descended from the truncate grandmother or truncate grandfather.

It is to be emphasized that the fertility of the truncates has been practically doubled (as determined by testing brother and sister) as a result of crossing out and extracting; and yet a marked degree of incompatibility exists between the gametes of the extracted truncates. For example, diagram C shows that the extracted male B produced 85.1 good sperm and the extracted female B produced 81.7 good eggs and yet the fertility as shown by testing

TABLE 6
Showing the effect on fertility of back-crossing the extracted truncates with their high-producing grandparents—the Woods Hole stock.
WH = Woods Hole. B = extracted truncate (F_2 extracted from truncate male); A = extracted truncate (F_2 extracted from truncate female); M = new wild stock captured at Woods Hole, Mass., used for control.

DATE	WH σ^6 \times B σ^6		WH σ^6 \times A σ^6		WH σ^6 \times WH σ^6		WH σ^6 \times B σ^6		WH σ^6 \times WH σ^6		A σ^6 \times B σ^6		A σ^6 \times B σ^6		M σ^6 \times M σ^6 (control)	
	Eggs	Adults	Eggs	Adults	Eggs	Adults	Eggs	Adults	Eggs	Adults	Eggs	Adults	Eggs	Adults	Eggs	Adults
Aug. 7.	40	36	44	5	20	17	43	33	20	12	70	33	41	18	51	51
8.	50	38	31	18	40	30	40	36	45	40	50	19	30	15	50	46
9.	51	43	60	42	50	43	45	39	37	26	50	38	50	26	60	60
10.	61	38	60	43	20	17	50	43	41	36	70	40	50	26	70	67
11.	50	33	76	66	60	52	60	50	56	47	65	50	52	29	66	61
12.	45	41	16	14	70	70	50	44	40	28	50	38	14	9		
13.	54	49	45	42	50	41	53	45	28	17	32	25	13	10		
14.	36	33	37	31	60	52	65	56	32	24	30	18	53	21		
15.	40	32	42	35	50	45	45	39	37	21	32	21	35	20		
16.	30	27	40	34	31	27	23	18	28	17	32	23	28	19		
17.	25	22	40	23	36	30	30	27	40	30	35	24	19	13		
18.	15	12	30	25	35	30	26	24	23	16	22	19	11	6		
19.	15	14	30	23	17	16	20	17	20	16	17	14	11	9		
20.	14	13	19	14	15	13	30	25	22	16	15	10	6	4		
21.	11	9	20	16	8	8	16	13	3	17	13	21	13	4		
22.	20	15	30	25	21	18	30	27	22	18	40	25	18	11		
23.	25	23	30	27	59	54	30	24	35	34	35	22	36	22		
24.	35	26	60	53	66	55	70	57	51	30	30	17	30	17		
Total	617	504	710	536	708	618	725	617	601	431	692	449	518	278	297	285
Per cent of fertility..	81.7	75.5	87.3	85.1	71.7	64.9	53.6	96								

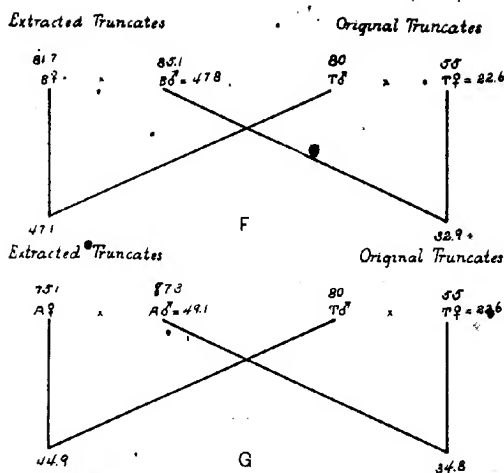


Diagram F Showing incompatibility that exists between truncates and extracted truncates (extracted from truncate ♂).

Diagram G Showing incompatibility that exists between truncates and extracted truncates (extracted from truncate ♂).

together was only 47.8. Diagram D shows practically the same condition.

As just stated the extracted truncates produce 81.7 good eggs and 85.1 good sperm (diagram C). We know from many former experiments that the truncates produce about 80 per cent of good sperm and 55 per cent of good eggs. Yet when these gametes are brought together, as shown in diagrams A and B there is a marked degree of incompatibility. Diagrams F and G based on this and former experiments, will show at a glance the facts that relate to incompatibility in this case.

It remains to be pointed out that there is a rise in fertility beyond that shown by either parent when the truncates extracted from the truncate grandfather B are crossed with the truncates extracted from the truncate grandmother A (diagram E). It seems strange that there should result a rise in fertility in this cross and also when the extracted truncates are back-crossed into their high-producing grandparental stock (diagrams C and D); and yet there results no rise in fertility when the extracted truncate female is back-

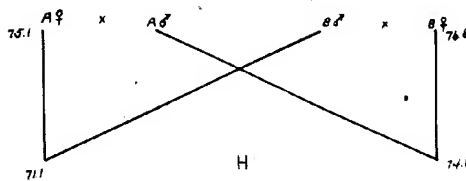


Diagram H Showing the fertility of the extracted longs when mated together and when crossed with each other.

crossed to the truncate male; and that in the reciprocal cross there is shown not only no rise in fertility but an actual incompatibility (diagrams A and B).

It has been shown that the fertility of the truncates is raised from about 25 per cent to 50 per cent by crossing out to a wild stock and extracting. It must be remembered, that the fertility of the wild stock from which the truncates were extracted was far below 100 per cent. It seems altogether probable that had a more fertile stock been used the fertility of the extracted truncates would have been still higher.

FERTILITY OF THE LONG-WINGED BROTHERS AND SISTERS OF THE EXTRACTED TRUNCATES

The question still remains as to how the long-winged brothers and sisters of the extracted truncates behave in respect to fertility when tested together. The data recorded shown in table 7 gives an answer to the question.

The results of this experiment as expressed in diagram H show that the long-winged brothers and sisters of the extracted truncates are more fertile than the extracted truncates (in this case by over 25 per cent). It would seem from this that while it is possible to put a certain amount of fertility into the gametes of the truncates, yet a certain degree of incompatibility remains with the truncate wing as evidenced by the fact that their long-winged brothers and sisters give a higher degree of fertility. It is to be noticed in this case that there is no rise in fertility on crossing. The fertility in the crosses is practically the same as in the controls.

EFFECT ON FERTILITY OF CROSSING THE TRUNCATES WITH THEIR LONG-WINGED BROTHERS AND SISTERS AND THE EFFECT ON FERTILITY WHEN BOTH OF THESE FORMS ARE CROSSED INTO THE WOODS HOLE STOCK

I wish to return to a consideration of the low fertility of the truncate stock and consider more especially the difference in fertility between the truncates and their long winged brothers and sisters. I shall also consider the behavior of fertility when the truncates are crossed to their long winged brothers and sisters, and also the behavior of fertility when both forms of the inconstant stock are out crossed to the Woods Hole stock. Table 8 shows the combinations made up, the number of eggs

TABLE 7

Showing fertility of the long-winged brothers and sisters of the extracted truncates when tested together and when crossed with each other. A = F_2 long-wing extracted from truncate grandmother. B = F_2 long-wing extracted from truncate grandfather

DATE 1913	A ♀ × A ♂		B ♀ × B ♂		A ♀ × B ♂		B ♀ × A ♂	
	Eggs	Adults	Eggs	Adults	Eggs	Adults	Eggs	Adults
August 15	30	21	40	26	65	51	50	31
16	31	23	30	21	45	33	50	42
17	50	35	35	19	40	24	40	23
18	30	17	36	30	27	18	41	35
19	20	13	44	33	22	18	29	22
20	23	18	19	15	29	17	30	22
21	13	7	40	33	25	20	31	15
22	49	36	27	23	60	41	45	37
23	40	37	70	58	41	28	57	42
24	80	60	71	59	60	55	60	48
25	50	37	63	50	50	39	40	29
26	50	35	86	73	70	50	53	47
27	40	38	52	35	55	35	45	37
28	50	34	60	39	50	37	50	38
29	40	32	40	39	50	25	51	37
30	47	40	16	13	40	24	25	16
Total	643	483	739	566	729	515	697	520
Per cent of fertility	75.1		76.6		71.1		74.6	

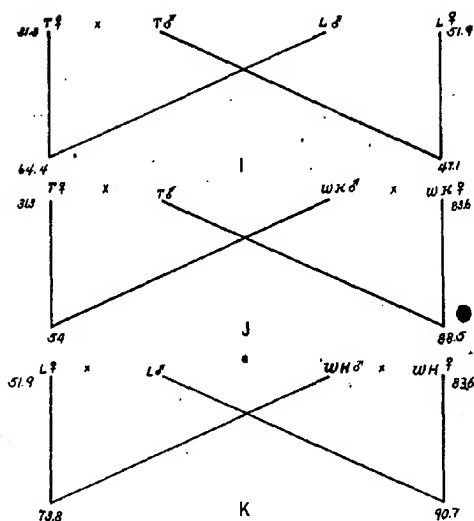
isolated day by day and the corresponding number of eggs that hatched.

The truncates in this experiment gave a fertility of 31.3 which is from 5 to 10 per cent higher than in any of the previous experiments. The Woods Hole stock shows a fertility of 83.6 which is much higher than that of previous experiments. The fact is that all the stocks at this time showed a rise in fertility. Whatever the meaning of this may be, the former relations hold as to the effect on fertility when crossed, as is evidenced by the fact that there is a marked rise in fertility when the truncate is crossed into the Woods Hole stock; compare diagram J with diagram C, Part II.

Diagram I brings out the effect of crossing within the truncate stock. The fertility of the truncates when tested together is 31.3 while that of their long-winged brothers and sisters when tested together is 51.9. When the longs are crossed to the truncates as the diagram shows, the high fertility of the longs is able to bring the fertility of the truncates up to its level and it would seem that in the case of the cross between the truncate female and the long male that there was a marked rise in fertility. The fact is that in this experiment this cross is higher by 10 per cent than in the case where the truncate female is tested with a wild male. I have made many crosses between the truncate female and the Woods Hole male with the result that the fertility of the combination stands almost invariably at 55 per cent. It would seem from this experiment and the evidence from former ones that the rise in fertility of this particular combination is significant.

A study of the diagrams will show a marked degree of incompatibility to exist between the flies with the long wings for while 51.9 of their eggs hatch when mated together yet no less than 90.7 of the sperm and 73.8 of the eggs are capable of entering into a combination that results in development, as is shown by crossing into the Woods Hole stock (diagram K).

The greatest degree of incompatibility however exists between the flies with the truncate wings for in this experiment they produced no less than 54 per cent of good eggs and 88.5 per cent of



Diagrams I, J and K Showing the effects on fertility of crossing within and without the inconstant truncate stock. T = truncate. L = long-wing thrown by truncates. WH = Woods Hole.

Diagram I Crosses between truncates and their long-winged brothers and sisters.

Diagram J Crosses between truncates and the Woods Hole stock.

Diagram K Crosses between the long-winged flies thrown by the truncates and the Woods Hole stock.

good sperm and yet only 31.3 of the eggs from the truncate females hatched when mated to the truncate males (diagram J).

It remains to be pointed out that a certain degree of incompatibility exists in the cross made between the truncate male and his long-winged sister. For their fertility when tested together is 47.1 and yet he is producing 88.5 fertile sperm and she is producing 73.8 fertile eggs as shown by outbreeding into the Woods Hole stock. In the cross between the truncate female and the long-winged male the incompatibility seems to be removed. For while the long-winged male is producing 90.7 fertile sperm the female is producing only 54 per cent of fertile eggs. Here, as I have

TABLE 8

Showing the effect on fertility of crossing the truncates with their long-winged brothers and sisters and the effect on fertility when both of these forms are crossed into the Woods Hole stock. T = truncate. L = long-wings from truncates. WH = Woods Hole stock

DATE 1913	1 T♂ × T♀ (CONTROL)		2 T♂ × L♀		3 L♂ × T♀		4 L♂ × L♀ (CONTROL)		5 T♂ × WH♀		6 T♀ × WH♂		7 L♀ × WH♂		8 L♂ × WH♀		9 WH♀ × WH♂ (CONTROL)	
	Eggs	Adults	Eggs	Adults	Eggs	Adults	Eggs	Adults	Eggs	Adults	Eggs	Adults	Eggs	Adults	Eggs	Adults	Eggs	Adults
Aug. 17	34	5	22	14			14	4	55	45	35	15	21	17	27	21	32	26
18	25	4	31	11	15	3	13	2	40	30	17	8	45	29		25	25	25
19	10	3	27	8	18	7	13	2	30	30	17	8	35	11	56	48	23	19
20	10	1	38	11	13	5	23	9	41	40	15	7	25	13	30	39	25	20
21	23	9	26	14	15	8	25	9	42	33	10	6	30	20	40	33	5	3
22	40	12	50	26	50	30	31	13	50	47	18	9	33	22	100	92	53	42
23	47	22	60	27	92	56	30	12	100	88	30	18	50	46	100	97	73	60
24	64	22	90	52	70	36	60	19	100	88	40	32	60	48	52	51	85	66
25	50	23	41	12	63	40	30	12	50	46	4	3	51	44	51	42	53	40
					55	37												
	56	23			52	31			61	37							60	51
	50	17	53	26	43	30	35	20	33	45	5	2	50	43	50	42	52	42
	27	40	34	27	40	33	42	23	60	56	5	2	17	12	40	40	50	43
	28	50	19	50	60	30	40	24	25	24	6	4	33	26	40	40	50	40
	29	57	10	47	45	27	37	50	40	32			8	7	44	36	50	44
	30	32	11	43	18	49	29	30	22	40	36		5	4	46	44	40	35
	31	30	10	30	16	28	16	34	24	34					25	20	40	33
Sept. 1	40	13	35	19	37	27	40	31	28	24					30	30	39	31
2	35	7	30	18	30	15	30	13	43	38					24	23	41	37
3	25	5	27	14	26	15	13	9	50	47					26	14	40	40
4	35	6	20	6	28	14	16	16	31	26					40	37	20	18
Total	735	236	754	365	829	534	543	282	979	866	502	109	469	346	815	739	856	715
Per cent of fertility	31.3		47.1		64.4		51.9		88.5		54		73.8		90.7		83.6	

pointed out, the incompatibility is removed and we have a distinct rise in fertility.

This evidence would seem to indicate that a marked difference in respect to fertility may exist between brothers and sisters of the same stock. In this case we find a great degree of incompatibility between the flies with a definite wing form—long-wings; the greatest degree of incompatibility between flies with truncate wings. We also find a marked degree of incompatibility when the truncate male is crossed to his long-winged sister but no incompatibility in the reciprocal cross.

ON THE EARLY PULSATIONS OF THE POSTERIOR
LYMPH HEARTS IN CHICK EMBRYOS: THEIR
RELATION TO THE BODY MOVEMENTS

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TWO CHARTS

INTRODUCTION

The lymph hearts of the chick were discovered by Budge in 1882. Previous to this, pulsating lymph hearts had been described in adult aquatic birds and in the ostrich and cassowary, by Stannius and Panizza. Budge found that lymph hearts, although absent in adult chickens, are present in the embryo and function from the ninth day to the time of hatching. He was able to inject these lymph hearts from the lymphatic vessels surrounding the allantoic arteries, in embryos of ten days and over, and he describes them as two rounded sacs, situated on each side, at the angle between the tail and pelvis, and superficial to the myotomes. He studied the muscles in the wall of the lymph heart by dissection of fixed preparations. Budge also discovered the lymph heart in living chicks, of eight days and over, and found that its pulsations did not coincide with those of the blood heart. Budge concluded that, since the lymph hearts make their appearance at approximately the same time as the lymphatic vessels of the allantois and atrophy with the disappearance of the allantois, they are intimately connected with the function of that organ.

A more extensive study of the lymph hearts of the chick was published in 1900 by Sala. The lymph hearts, according to Sala, in embryos of eight days, are clear almond-shaped sacs situated on either side of the tail, lateral to the myotomes and somewhat ventral to the caudal margin of the os ilii. After the

tenth day, the hearts grow more rounded in shape and become surrounded by a layer of fat. With the gradual reduction of the curve of the vertebral column, they eventually occupy a more dorsal and anterior position with reference to the os ilii. Sala found that the lymph hearts communicate with the intersegmental coccygeal veins—at first connecting with the first five of these veins, and, in later stages, with three of them.

From his study of the lymph heart in cross sections, Sala found that, in embryos of seven and one-half days, its walls are made up of mesenchyme which penetrates the lumen of the sac in the form of trabeculae. In nine-day chicks, muscle fibers begin to differentiate in this connective tissue, and eventually they form a layer in direct contact with the endothelial lining. In embryos of thirteen days the muscle fibers and the endothelium are separated by a layer of mesenchyme.

Sala also investigated the earlier stages of the lymph heart, in cross sections of chicks six and one-half to seven days old. He found a row of irregular spaces connected with the first five intersegmental coccygeal veins and concluded that the lymph heart was formed by the flowing together of these spaces.

Mierzejewski in 1909 studied the superficial lymphatics of chick embryos by injection. He confirmed the results of Sala as to the development of the lymph heart but states that the anlage of the lymph heart first appears in chicks of five and one-half days, instead of six and one-half days, as Sala had stated. Mierzejewski agrees with Sala as to the time of appearance of the muscle fibers in the wall of the lymph heart and states that, at nine days, the lymph heart begins to pulsate and beats once to every eight or ten contractions of the blood heart.

The authors have studied this region in the stages of embryos investigated by Sala and Mierzejewski and also in earlier stages. We have found that the spaces in the mesenchyme, described by Sala as the anlage of the lymph heart, are in reality definite enlarged lymphatic capillaries, which form a continuous plexus connected with the intersegmental coccygeal veins, and whose endothelial lining can be demonstrated easily by injection with dilute silver nitrate, and also by the microscopic study of sections.

At a much earlier stage—in chicks of four and one-half to five days—lymphatic vessels are present, although their size is much smaller, and in embryos of five to six days, the lymphatic plexus, which precedes the definite pulsating lymph heart, normally contains stagnant blood, which enters it through its connections with the intersegmental veins of the tail. Preliminary reports of studies made of this lymphatic plexus in living embryos and by the method of injection, have been published. A more complete account of these researches, together with careful microscopic studies of the endothelium is soon to be published.

In the course of this study of the developing lymph heart, we noticed, while examining a chick of seven days under the binocular microscope, that there occurred several beats of the lymph heart simultaneously with vigorous movements of the tail. As muscular contractions of body, tail and limbs had previously been seen in embryos of various ages, there was suggested a study of the early pulsation of the lymph heart in relation to these movements.

For these studies we used a binocular microscope, enclosed in a warm chamber which was heated by an electric light bulb or a small alcohol lamp. An opening was made in the shell and the shell membrane over the region of the embryo, was carefully removed. Throughout the observation, the embryo was kept moist by the occasional addition of a few drops of Ringer's solution, warmed to 38 to 39°C. When the eggs were opened in this way, the chicks remained alive and healthy for from five to eight hours.

THE MUSCULAR MOVEMENTS OF CHICK EMBRYOS

When an egg, incubated four days or more, is opened in this manner the embryo is seen to be undergoing violent movements. These movements are found to belong to two types: one in which the embryo as a whole is moved, with a continuous back and forth swaying motion around the umbilical cord as a center, and the other type, in which the movements are spasmodic and affect various parts of the embryo. The first type is caused by the peristaltic contractions of the amnion, described by von Baer in 1828.

Von Baer observed movements in chicks of five days which he believed to be due to the exposure of the embryo to the cold air. On the seventh day he states that the movements have become more general: "Der Embryo schwingt in Amnion hin und her, auf dem Nabel, wie auf einem befestigten Stiele—Es schien mir daher eine Art unregelmässiger Pulsation im Amnion." Von Baer found that the contractions of the amnion were very active in chicks of eight or nine days and that after that time they became weaker.

The subject of amniotic contraction was further investigated by Remak in 1854. After opening the egg, he noticed violent wave-like movements of the amnion, which were now and then followed by quieter rhythmical contractions. When the contractions ceased they could be started again by pricking the amnion with a needle. Pieces of amnion, examined under a simple lens, were found to contract when stimulated. Remak found smooth muscle fibers in the amnion, which increased in abundance up to the tenth day of incubation; after this time their number diminished. Nerves, according to Remak, are not present in the amnion. Like von Baer, Remak doubted the presence of these peristaltic contractions in normal embryos inside the egg, and stated that the violent contractions were probably caused by exposure to the air.

This last point was refuted by Vulpian in 1857, who observed the embryo in unopened eggs held before a bright light. He saw the swaying movement of the embryo in chicks of five to eight days and found it to be violent and continuous in the unopened eggs, under normal conditions. He also described contraction of the under surface of the allantois in chicks of twelve days and over.

Preyer in 1885, studied these contractions of the amnion, as well as the other type of movements in chick embryos. He studied chicks on each day of their development, by means of a specially constructed ooscope, both before and after opening the shell and found that the amniotic contractions first appeared in embryos of four days, and continued up to the thirteenth day of incubation. At this time, the violent swinging of the

embryo becomes a gentle swaying and, in later stages, all movement is absent. Preyer showed that the amniotic contractions, contrary to the ideas of von Baer and Remak, were not caused by the cold air, but were normally present in warm eggs and ceased a short time after exposure of the egg to cold air.

Preyer's method enabled him to keep the embryos alive and active for only a short time after opening the shell. By making use of the warm chamber and the binocular microscope, we have been able to watch the passive and active movements of the chick for several hours after opening the shell, and to study them in greater detail. We found that the amniotic contractions follow each other in rapid succession: each peristaltic wave consumes on an average, 3.3 seconds, and about 4.5 seconds elapses between the cessation of one wave and the beginning of the next one. We also observed, in a five-day chick, four distinct types of amniotic contraction: (1) The peristalsis starts at the top of the head and passes completely down to the tail of the embryo; (2) The contraction starts at the same place but extends only half as far, i.e., to the posterior edge of the wing; (3) The wave starts over the neck bend, half way between the ear vesicle and the anterior edge of the wing and extends simultaneously in both directions; (4) The contraction wave starts posteriorly and extends from the tail to the head end of the embryo. Types (1) and (4) were found to occur most frequently, and in type (1) the passive movement of the whole embryo was the greatest. With this type there is evidently a rapid circulation of the amniotic fluid for as the contraction wave passes posteriorly, the fluid is squeezed out and the amnion comes in contact with the embryo, and the amount of fluid increases in the relaxed part of the sac. The extremities are pressed tightly against the body wall, as the amniotic wave passes over them. This undoubtedly has an effect on the form of the chick, for in embryos which had lived for several hours with the amnion removed, we noticed that the extremities, on the side away from the yolk, showed a tendency to grow away from the body until they often projected at right angles to it.

The other type of movements—the active contractions of the embryo—have also been recorded by many observers. Preyer, in an extensive review of the older literature on the subject, mentions that such observations were made by Harvey in 1651, by Béguelin in the middle of the eighteenth century, and by Home in 1822, as well as by von Baer and Remak. Most of these observers, however, did not distinguish between the passive movements of the embryo, caused by the contraction of the amnion, and the active movements of the chick itself. However, Dareste, in 1879, noticed movements in an embryo without an amnion, and thus demonstrated conclusively the independence of this second type of movement.

Preyer gives an interesting and comprehensive account of these active movements in chick embryos. From the first to the fifth day of incubation he found no embryonic movements, aside from the beating of the heart. Mechanical and electrical stimulation of the embryo produced no effect. On the fifth day, Preyer first observed the active embryonic movements and the amniotic contractions. The first movements of the embryo, according to Preyer, are simple in character and consist chiefly in a bending of the back from side to side. The head, tail, and extremities are moved passively at this stage. In successive later stages, the movements become more numerous, violent and complicated, as the various parts of the embryo differentiate. At the sixth and seventh day, the bending of the body becomes more pronounced, the tail contracts independently, the head nods, and the paddle-like extremities are moved inward and outward, and from the eighth to the eleventh day, the movements continue to increase in strength and variety. Intervals occur at all these stages, during which the embryo does not move. During this second period of development—from the fifth to the eleventh day—Preyer found no response of the musculature to mechanical stimulation or to injury, such as amputation of a leg. However he occasionally observed a weak response of certain muscles, following electrical stimulation of the spinal cord, on the ninth and tenth day of incubation.

In the third stage of development described by Preyer—the period from the eleventh to the fifteenth day—the active movements of the embryo continue to occur, at frequent intervals, and, in addition to those present in younger chicks, movements of the individual toes and opening and closing of the eyelids and beak occur frequently. At this stage, reaction to mechanical stimulation is doubtful: at times a seemingly definite response is obtained and, at other times, the embryo remains motionless when stimulated, although continuing to move spontaneously. At this stage, however, chicks responded definitely to electrical stimulation, by movements of the limbs, toes, beak, etc., according to the point at which the stimulus was applied.

In the last period of development— from the sixteenth to the twentieth day of incubation, Preyer states that spontaneous movements are much less frequent, but that the chicks respond readily to electric stimulation by tetanic contraction, and show an increased tendency to respond reflexly to mechanical stimulation.

With his especially constructed ooscope, Preyer was able to observe, at all stages, both types of embryonic movements, those due to the contractions of the amnion, and those due to contractions of the muscles of the embryo itself, in the unopened egg, kept at incubator temperature, thus proving that they are normal, and not caused by outside factors introduced at the time of opening the shell.

Our observations of the muscular movements of chicks were confined to embryos of four to fourteen days, since this comprises the important periods in the development and early functioning of the lymph heart. Our observations agree with Preyer's description of the character of these movements, and of their first appearance in chicks of four days. However, in addition to the movements of the various parts of the embryo, described by Preyer, we noticed that sudden violent jerks, which seemed to involve the entire musculature of the embryo, occurred at all of the stages studied. With regard to the absence of all reaction to mechanical stimulation, in chicks between the fifth and eleventh day, our results are in entire agreement with those of Preyer.

One very striking characteristic of the active movements of chicks during the period of their development from four to fourteen days, is their periodicity. The movements occur in definite groups or spasms followed by an intermission or period of rest, after which the movements recur. This periodicity was not noted by Preyer or by earlier observers, although they record the fact that the active movements were not continuous.

While the active movements of the embryo can be observed with the amnion intact and have been studied through this membrane, they are more easily observed when it is cut across and drawn aside. In observing the periodic movements, we kept records of the minute and second at which each spasm commenced, its duration, and the interval from the beginning of one spasm to the beginning of the next. We found that the duration of the periodic movements and the periods of rest varied in embryos of different ages. For example, in a chick of six and one-half days, the duration of the periodic spasms averaged 13 seconds and the period of rest 39 seconds, while in an embryo of nine days, the movements were found to continue for 28.5 seconds and to be followed by an average period of 54.5 seconds, in which the chick did not move. In all the stages studied, one or two movements of the tail, a slight twitch of the leg or wing, or a single jerk of the whole body occasionally occurred, apart from the periodic spasms. The interval from such an isolated movement until the beginning of the next was always shorter than the interval between the true periodic spasms.

We also made some observations of the reaction of embryos to changes in temperature. When the temperature of the warm chamber was raised until a small thermometer placed near the embryo registered 41° , the active movements ceased, after a few minutes, and the chick remained motionless until the temperature was lowered to 38 or 39° , when the periodic movements were resumed. We also found, as Dareste had observed in the case of a chick without an amnion, that the muscular movements ceased when the temperature was lowered to 35° or under and began again when the temperature was raised. When the amnion was left intact, the response of the body movements to

a change of temperature was delayed. In this case, when the temperature was raised to 41° or lowered to 35° , the peristaltic contractions ceased and the amnion relaxed, allowing the embryo to sink deep into the yolk. The periodic movements persisted for several minutes, in such instances, probably owing to the more sheltered position of the embryo.

The movements of fish and amphibian embryos in the egg have been studied by many investigators. Preyer reviewed the literature on the subject and published many new observations on the embryos of the trout and the frog. He finds both active and passive movements occurring in these embryos: the passive movements are due to the almost continuous rotation of the embryo inside the egg, caused by the action of the cilia. The active movements, according to Preyer's description, resemble greatly those present in chick embryos of four to five days, the body bends from side to side and the head and tail bend at the same time. More recently, Harrison, Hooker, Wintrebert and Coghill have observed movements in early amphibian embryos, and Paton has studied them in embryo fish. No periodic movements were noted by any of the investigators of amphibian material. Mention is made of spontaneous movements, but as a casual and not a frequent occurrence. Harrison distinguishes two types of response to stimulation in young amphibian embryos: (1) the non-nervous type, characterized by a sharp tonic contraction and (2) the nervous type which resembles a swimming movement. The former has been shown to be the more primitive, since it is noticed in embryos before the peripheral nerves have developed (Wintrebert) and has also been demonstrated in embryos in which the spinal cord had been cut or removed (Harrison and Hooker). It appears, then, that the early movements of chick embryos differ from those noted in amphibia, by occurring periodically and with greater frequency, and also in the fact that they can not be instigated or altered by mechanical stimuli, such as pressure or direct piercing of the muscles with glass needles.

The early movements of fish embryos, described by Paton show a closer resemblance to the movements of chicks. In

Selachian embryos of 3.5 to 4 mm., according to Paton, jerky lateral movements begin. These are rhythmic in character: several movements occur, followed by a pause of several seconds and then the movements begin again. They do not appear to be affected by mechanical stimulation of the embryo, although they are increased by squeezing the yolk sac. The rhythm of the movements is altered by heat, by brilliant light, and by interfering with the normal swing of the head from side to side. In all of these cases, the movements become more violent. In the case of approaching death, the contractions become tonic. Fish embryos appear to resemble chicks in the periodic character of their early movements and in their failure to respond to mechanical stimuli.

In Mammalia, Preyer (pp. 586-595) studied the movements in guinea-pigs. The earliest movements were seen in a 'fourth week' embryo of 16 mm. which "bewegte den Rumpf in situ stark." In this embryo, he notes "Zehen noch nicht getrennt." In all embryos of successive weeks, movements were seen, increasing in intensity. In the 16 mm. embryo electrical stimulation over the spinal cord caused muscle contractions, and in 26.4 mm. embryos peripheral stimulation, both electrical and mechanical, led to reflex movements.

That periodic muscular movements, beginning at least as early as the fifth day of incubation and continuing with increasing intensity throughout the remainder of incubation, take place in developing chicks (and that movements occur in mammalian embryos as well, beginning at least as early as 16 mm. in guinea pigs—Preyer) is a fact of much significance in the study of the physiology of the embryo and of the mechanical factors concerned in development, especially of muscles, bones and joints—a fact which has been frequently overlooked.

Thus Thoma, in his study of bone growth, takes up the mechanical factors acting on cartilage at the time of beginning ossification. On page 350 he says: "Als Ursache der Belastung kann demgemäss im wesentlichen nur die Spannung der Hautdecken und der Muskulatur." No mention is made of the tension caused by contraction of the muscles.

Again R. Schmidt, p. 95, in discussing the ossification of bones in embryos, concludes "Da die Grundzüge der Architektur sich ausbilden, ehe noch die Muskelemente kontraktile sind (Hencke, Bernay, u. a.) kann die Ursache nicht unmittelbar in Gebrauch zu suchen sein." Herbst (p. 7), after quoting the above, adds "so schliesst Schmidt ganz richtig." The tension produced by muscle contractions, left out of

account by these observers, must be quite considerable, since, according to our observations, chicks of six days move very actively, approximately one quarter of the time, a total of six hours out of the twenty-four, chicks of nine days, one-third of the time or eight hours out of the twenty-four, while up to the thirteenth or fourteenth days the time of the movements increases still further.

Harrison, on the other hand, has made a significant beginning at studying the relation between muscular movement and the development of muscles and bones. He found that muscles differentiate relatively normally in tadpoles whose movements are prevented by the use of chloretone, as well as by the removal of nerves. He also observed that ossification occurred in the hind limb of a tadpole into which no nerve had grown, and hence in which there was no muscular movement.

In a new-born calf E. H. Weber found total absence of the spinal cord and nerves below the first thoracic vertebra. Corresponding to the absence of nerves, the muscles were entirely absent, in the posterior part of the body, and while the bones of the hind limb were present and described as 'normally developed' yet the hind limbs weighed only one-half as much as the fore limbs, no description is given of their architecture, and all the joints were ankylosed. Between these two sets of observations there is a wide gap in which it remains to be determined *to how great an extent* the muscular movements in embryos regulate the development of the typical architecture of bones, the form of joint surfaces and the growth of muscles.

THE PULSATION OF THE LYMPH HEART

In our observations of the pulsation of the lymph heart, in chicks of various stages, we kept careful records of the exact time at which each spasm of muscular movements commenced, the length of the interval between movements, and the number of lymph heart beats occurring during the observation.

- As already stated, in chicks of 5 to 6 days, the region in the angle of the pelvis and tail, later occupied by the lymph heart contains a plexus of lymphatic capillaries filled with stagnant blood. At this stage, the movement of the tail from side to side, at the time of each periodic spasm of body movements, is violent enough to cause a blanching of this whole region, particularly noticeable in the superficial blood capillaries. But the definite localized contractions so characteristic of the beating lymph heart of later stages, are not yet present.

Stage 1. In embryos of 6 to 7 days (measuring from 19 to 22 mm. greatest length, before fixation) the stagnant blood is

no longer present in the plexus of the posterior lymph heart region. In the living chick, this area has a swollen, translucent appearance and, upon injection, a plexus shows up, which is more compact and which is composed of fewer and larger vessels than the lymphatic plexus of younger embryos. At this stage, distinct localized pulsations of this clear area are noticeable in living chicks. Physiologically, then, a posterior lymph heart is now present.

Embryos of this stage were kept alive and under continuous observation for from 2 to 5 hours and records made of every lymph heart contraction and of each periodic spasm of body movements. It was found that the lymph heart beats occurred invariably at the time of the periodic spasms and never in the intervals between these body movements. Moreover we found that, at this stage, a beat of the lymph heart was always accompanied by a movement of the tail. Tail movements occurred, unaccompanied by any lymph heart contraction, but beats of the lymph heart unaccompanied by a tail movement were never observed.

After an embryo of this stage had been observed for an hour and this relation of lymph heart beats to the periodic body movements recorded, chloretone (1 : 4000 in Ringer's solution at the temperature of the chick) was dropped on the embryo. A minute later the body contractions ceased and the chick remained motionless for a period of fifteen minutes. During this time no pulsation of the lymph heart was observed. As the effect of the anaesthesia wore off the spasmodic contractions returned and soon regained their former periodicity. The lymph heart beats returned at the same time and accompanied the body spasms as before and each beat was always associated with a contraction of the tail. It is evident, then, that at this earliest stage in which definite pulsations of the posterior lymph heart are visible, the beating is inseparably connected with the periodic movements of the embryo (charts 1 and 2: Stage 1).

We found, however, that unlike the rest of the musculature of the chick, the lymph heart muscle responds to direct stimulation and always contracts when it is punctured. In chicks of

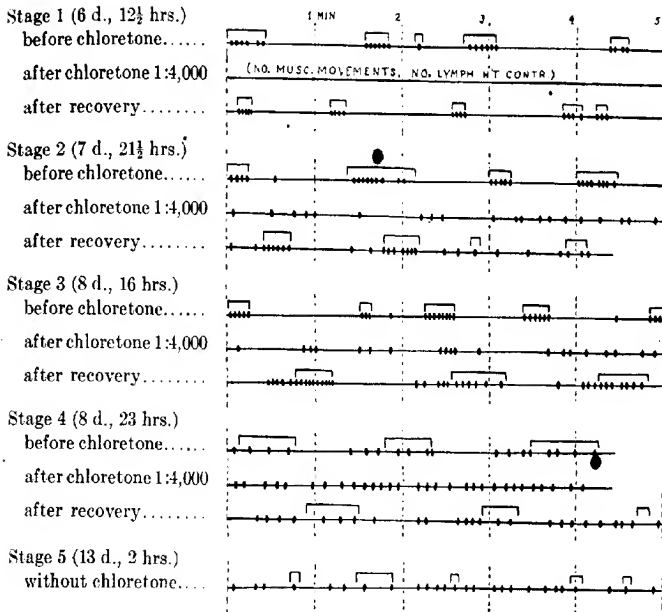


Chart 1 Shows graphically the relation between the lymph heart beats and body movements in different stages, before and after chloretone and after recovery from chloretone. The brackets — indicate the duration of the periods of body movement, the spaces between the brackets are the intervals of rest, and the dots are lymph heart pulsations. Selected portions of actual records are reproduced. Spaces between successive beats, length of periods of body movements and of intervals of rest represent actual proportional time intervals.

six days, in which the lymph heart pulsations, although distinct, are not yet very strong, the lymph heart contracted only once when stimulated directly by puncture with a glass needle. In the older embryos of Stage 1, puncture of the lymph heart caused it to beat six or eight times in succession and these beats were accompanied by tail and body movements. If the chick was anaesthetized at the time of puncture, each beat was accompanied by a slight mechanical pulling of the tail.

This early stage of the beating lymph heart, in which the pulsations occur only at the time of the periodic spasms of body

	OBSERVATION TIME	AVERAGE DURATION OF PERIODS OF BODY MOVEMENTS	AVERAGE TIME PERIODS DURING WHICH ONE MOVING PERIOD TO BEGINNING OF NEXT	AVERAGE NUMBER OF BEATS WITH Lymph HEART IN EACH PERIOD OF MOVEMENT	AVERAGE NUMBER OF Lymph HEART BEATS IN EACH INTERVAL OF REST	AVERAGE NUMBER OF Lymph HEART BEATS PER MIN. UTR
Stage 1 (6 d., 12½ hrs.) (GL. 20 mm.)						
before chloretone....	45' 52"	13"	54"	3.3	0	3.8
after chloretone.....	15'	none			0	
after recovery.....	16'	11"	1' 06"	3.8	0	3.3
Stage 2 (7 d., 21½ hrs.) (GL. 24½ mm.)						
before chloretone....	1° 12' 50"	20"	1' 06"	5.3	1/9 ⁽¹⁾	5.1
after chloretone.....	7' 20"	none				4.7
after recovery.....	20' 10"	not rec.	1' 18"	5.6	1.8	5.1
Stage 3 (8 d., 16 hrs.) (GL. 27 mm.)						
before chloretone....	37' 50"	not rec.	1' 13"	6.3	4/5 ⁽¹⁾	6.7
after chloretone.....	11' 22"	none				4.3
after recovery.....	17' 34"	not rec.	1' 57"	6.8	5.6	6.8
Stage 4 (8 d., 23 hrs.) (GL. 29 mm.)						
before chloretone....	16' 28"	28.5"	1' 23"	3.4	2.6	4.5
after chloretone.....	18' 12"	none				8.0
after recovery.....	7' 8"	19"	1' 31"	not rec.	5.0	5.0
Stage 5 (13 d., 2 hrs.) no chloretone.....	18' 51"	not rec.	1' 08"	1.6	3.4	4.8

Chart 2● Shows the results of estimations as to average duration of periods of movement, etc., before, during and after the use of chloretone, at the various stages. The records used for this chart are the ones from which the selections in chart 1 were made: (1) (Stage 2) means that approximately one lymph heart pulsation occurred in each nine periods of rest; (1) (State 3) means, similarly that in each five periods of rest four single lymph heart pulsations occurred.

movements (unless the heart is actually punctured), was not observed by Budge, Sala, or Mierzejewski. These investigators all state that no contractions of the lymph heart occur before the ninth day of incubation. In all probability, this is due to the fact that the eggs, which they used, were opened in water and submitted at once to unfavorable conditions of temperature. In such a case the periodic movements of the embryo would cease

at once, and, with them, the pulsations of the lymph heart in chicks of this stage.

In chicks of seven to seven and one-half days (measuring 22 to 24 mm.) our records show practically the same connection between the beating of the lymph heart and the body movements. The lymph heart beat from three to nine times during each periodic spasm and never in the intervals of rest. But, at this stage, strong lymph heart contractions were accompanied at times by feeble tail movements and occasionally they were dissociated altogether from contractions of the tail.

Stage 2. Our observations of chicks of eight days showed that, on the average, 5.3 beats of the lymph heart accompanied each periodic spasm but that the majority of these beats were dis-coordinated from the tail movements. In addition, an occasional single pulsation of the lymph heart occurred in the period between spasms, with no accompanying body movement. Injections of the lymph heart at this and later stages, show it to possess the form of a definite oval sac.

One embryo of this stage (7 days, 21½ hours, measuring 24.5 mm.) was kept under continuous observation for four hours, and showed an interesting reaction to the treatment with chloretone. The chick was first observed for an hour and a half. We found that an average of 5.3 lymph heart beats accompanied each spasm and that, occasionally, an independent lymph heart contraction occurred in the period of rest. Chloretone (1:4000 in warm Ringer's solution) was then dropped on the embryo. The body movements and lymph heart beats both ceased for a few minutes. Then the lymph heart began to beat, an average of 4.7 times in every minute, with no accompanying body movements whatever. This beating of the lymph heart continued for forty minutes before the effect of the chloretone wore off. With the elimination of the body movements, the lymph heart pulsations occurred singly, instead of in groups, and they lost all semblance of periodicity.

Finally, after about fifty minutes, the body movements began to recur, gradually regaining their former periodicity. With the return of the body movements, the lymph heart regained its

former habit of beating approximately five times during each periodic spasm. However, it did not return completely to its previous mode of functioning for it still continued to contract from 2 to 4 times, independently, at irregular intervals during the period of rest. The observation was continued for an hour after the return of the body movements to their normal periodic rhythm and the lymph heart continued to contract in this same manner (charts 1 and 2: Stage 2).

Stage 3. Chicks of eight and one-half to nine days (27 to 29 mm.) resembled those of Stage 2, except for a slight increase in the independence of the beating lymph heart. An average of six pulsations took place periodically at the time of the body movements, but an isolated beat occurred in nearly every period of rest. Chlorotone destroyed the periodicity of the lymph heart beats, which then occurred irregularly, about four or five to a minute. After the recovery from the anesthesia and the return of the body movements, the lymph heart again averaged six beats during each periodic spasm. In addition, it contracted about five times in every period of rest and these isolated beats, as a rule, preceded the period of body movements (charts 1 and 2: Stage 3).

When an embryo of Stage 3 or 4 was anesthetized and the lymph heart punctured by a fine needle, the lymph heart contracted six or eight times in succession, with no accompanying tail or body movements.

Stage 4. In chicks of nine to nine and one-half days (29 to 30 mm.) we found that the lymph heart still showed a tendency to beat periodically, but that its rhythm had now become somewhat discoordinated from that of the body movements. Although the periodic spasms in this stage were as long as or longer in their duration than those of younger embryos, the number of lymph heart beats occurring at this time was found to be less, an average of three beats instead of six. The number of isolated single beats occurring between spasms, however, was increased to three or four per interval. The isolated beats, as a rule, preceded the periods of body movements and, immediately after each spasm, there was a period of rest in which neither

body movements nor lymph heart beats were observed. When the body movements were paralyzed by chloretone, the groups of periodic lymph heart beats ceased and an increased number of single pulsations occurred, at irregular intervals. There were approximately eight beats per minute (charts 1 and 2; Stage 4).

Stage 5. Finally, in embryos of 10 to 13 days, the lymph heart contractions were found to be quite independent of the periodic body movements. At this stage, there was no semblance of periodicity in the pulsations of the lymph heart. Beats were seen to occur during the periodic spasms, but the intervals between such beats were not shorter than between those occurring in the period of rest. On the other hand, we observed several spasms during which no lymph heart beats whatever occurred (charts 1 and 2; Stage 5).

After the recovery from chloretone in Stages 2, 3 and 4, although the isolated number of heart beats was always greater than those occurring before the anesthesia, the total number of beats per minute remained practically the same. Also the number of beats per minute occurring during the period of anesthesia was not materially changed from the number originally present, in each of these stages (see chart 2). Although the chloretone dissociates the lymph heart pulsations from the body movements and greatly alters the rhythm in Stages 2, 3 and 4, it does not change, to any noticeable degree, the total number of lymph heart beats. Stage 1 is the only stage in which the function of the lymph heart is seriously interfered with, by the addition of chloretone, for in this stage only is it inseparably connected with the functioning of the voluntary muscular apparatus.

SUMMARY

We find, then, that pulsation of the lymph heart first appears in embryos of six to seven days (19 to 22 mm.) and that, at this earliest stage, the beating of the lymph heart is intimately connected with the periodic muscular movements of the embryo. Lymph heart contractions accompany the periodic spasms, never occur in the interim between body movements and each beat is invariably accompanied by a contraction of the tail. When

the body movements are paralyzed by chloretone, the lymph heart pulsations also cease.

In successive later stages, we find a gradual increase in the independence of the beating lymph heart. First, the pulsations become dissociated from the tail contraction, although still occurring only during the periods of body movements. Then we find a series of stages in which the lymph heart contracts more and more frequently during the period of rest, although still beating a number of times during each spasm. When chloretone is added, at these stages, a larger number of single pulsations occur independently, at irregular intervals. At these stages, the lymph heart is capable of entirely independent function, but is influenced, in its rhythm by the periodic spasms of body movements.

Finally, a stage is reached in which the lymph heart pulsations are uninfluenced, in any way, by the body movements. During all of these stages, mechanical stimuli, such as pressure over the surface with a fine needle, or direct puncture of the myotomes, failed to influence the body movements. Although the lymph heart does not respond to pressure over the surface, it always contracts when its wall is actually pierced. At the stage in which the lymph heart is not yet independent, such a puncture instigates tail contractions along with the lymph heart pulsations, while at later stages it stimulates the lymph heart alone.

DISCUSSION

In connection with our observations of the early pulsations of the lymph heart, we made serial sections of all the stages studied. In every case, the embryo sectioned was one which had been observed in the living and in which the movements and lymph heart pulsations had been recorded. From the study of these cross sections, however, we were unable to discover a definite anatomical relationship corresponding to the close functional association which exists, at first, between the lymph heart and the body musculature.

The following possibilities have suggested themselves in the course of our observations. It is possible:

1. That the lymph heart musculature is derived from the myotomes and that there is for a time a connection between the two.
2. That the muscle of the lymph heart is temporarily supplied by the same nerves which supply the myotomes.
3. That the lymph heart is merely stimulated mechanically by the contraction of the nearby muscles.
4. That, at first, the contractions of the lymph heart result from the same stimulus which brings about the periodic body movements, and that this stimulus is gradually subordinated to the stimulus caused by the increasing amount of lymph, brought to the lymph heart by the lymphatic vessels from the fast growing allantois and from the posterior half of the chick.

As regards the derivation of the musculature of the lymph heart from the myotomes, we have been unable to reach a definite conclusion from sections alone. In five-day chicks, the ventral extension of the myotome lies medial to the lymph heart plexus, separated by two or three layers of mesenchyme cells. During the succeeding day, this lateral portion of the myotome, which lies between the dorsal and ventral parts, becomes thinner, and in chicks of seven days and a few hours, only a few small bundles of somewhat isolated muscle cells remain. In sections of embryos of six and one-half days, with the oil immersion lens, we have seen muscle fibrillae in the wall of the lymph heart, which, at this stage, still retains the form of a plexus of lymphatic vessels. The muscle of the lymph heart, therefore, is present at an earlier stage than was supposed by Sala, who asserts that only mesenchyme is visible around the sac in embryos younger than eight and one-half days. In chicks of six and one-half to seven days, which is the stage at which the lymph heart beats always synchronously with the body movements, the wall of the lymph heart lies in close proximity to the thin lateral portion of the myotome already referred to. But, as regards the presence of a definite connection between the muscle fibrillae in the wall of the lymph heart and those composing this transitory portion of the myotome, we were unable to reach a positive conclusion.

In the matter of a common nerve supply for the myotomes and the lymph heart, our study of sections yielded only negative results, since we were unable to find any nerves in the wall of the lymph heart at any of the stages studied. However, further study with special nerve stains is necessary before this possibility can be excluded altogether.

We have already mentioned the fact that, during the earliest stage of its pulsation, the lymph heart lies in close proximity to the transitory lateral portion of the myotome. It is quite possible, therefore, that the contraction of the myotome, at this earliest stage, might stimulate the muscle of the lymph heart to contract, merely mechanically, without the presence of an actual connection between the two. In chicks of eight to nine days, with the disappearance of this part of the myotome, the lymph heart comes to occupy an isolated position in the mesenchyme. At this stage, however, the pulsation of the lymph heart is still influenced in its rhythm by the periodic contractions of the embryo. In an embryo of eleven days, the lymph heart has shifted its position and it now lies in close proximity to the dorsal muscle. But in this stage, in spite of its situation, the lymph heart is entirely independent in its beating, from the contraction of the rest of the musculature. Therefore, although the phenomena of the earliest stage of the lymph heart pulsation might be accounted for by the proximity of the myotome, which by its contraction stimulates the muscle of the lymph heart directly, the gradual manner in which the lymph heart pulsation becomes dissociated from the body contractions cannot be explained in this way. For, unlike the change in their physiological relationship, the change in the relative position of the myotome and the lymph heart muscle is sudden and not gradual.

In injecting the superficial lymphatics in living chicks, we have often observed that the lymph heart contracted as the fluid entered it. It is therefore capable of reaction to a stimulus from within as well as to the mechanical stimulation of its wall, and the gradually increasing independence of the lymph heart pulsations from the contractions of the body musculature may be

due to the growing intensity of the stimulus from within, occasioned by the constantly increasing flow of lymph. But further histological and experimental investigations are necessary for the complete explanation of the early pulsations of the lymph heart in chick embryos.

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STUDIES ON THE PHYSIOLOGY OF REPRODUCTION
IN THE DOMESTIC FOWL. VIII. ON SOME PHYSI-
OLOGICAL EFFECTS OF LIGATION, SECTION,
OR REMOVAL OF THE OVIDUCT¹

RAYMOND PEARL AND MAYNIE R. CURTIS

For several years there have been in progress at this Laboratory experiments dealing with various phases of the physiology of egg production. Among the reports of these investigations already published are several which deal with the physiology of the oviduct. A few of the more important of the results already obtained may be reviewed briefly here, in order to show more clearly the relation to them of the present findings.

1. Albumen secretion is not confined, as was formerly supposed, to the albumen secreting region of the oviduct, but also takes place in the isthmus and uterus. It is in fact probably a general function of the entire oviduct (Pearl and Curtis '12, and Surface '12).

2. There is some good evidence that for any part of the oviduct the effective stimulus to secretion is mechanical. Tarchanoff ('84) and Weidenfeld ('05) showed that a normal set of egg envelopes including chalazae, albumen, egg membrane and shell may be formed around an artificial yolk (wood, rubber, or amber bead). This result has been confirmed at this Laboratory with artificial yolks of agar-agar. Our experiments on this point have never been described in detail. The results were briefly stated in a previous paper (Curtis '14b). These experiments also agree with those of Tarchanoff that this result does not always follow the introduction of an artificial yolk into an active oviduct. The other necessary conditions

¹ Papers from the Biological Laboratory of the Maine Agricultural Experiment Station, No. 68.

are not yet fully understood. Pearl and Surface ('09 a) published a short report of a preliminary investigation of the stimulus which sets up the shell secreting activity. They showed that when the intestine was anastomosed to the oviduct above the uterus the faeces extruded through the vagina or retained in the uterus were covered with a deposit of shell material. They concluded that the immediate effective stimulus to shell secretion is mechanical and that shell formation is a local reflex not dependent upon a specific activity of other parts of the reproductive apparatus.

3. The amount of secretion by the duct depends in part upon the intensity of stimulation. Within the eggs of the same individual the weight of albumen is highly correlated with the weight of the yolk and the weight of shell is significantly positively correlated with the weight of both albumen and shell (Curtis '14 a). In yolkless, single, double and triple-yolked eggs the weight of albumen and shell are directly related to the number of yolks contained (Pearl '10, and Curtis '14 b).

4. The muscular activity of the walls of the oviduct are undoubtedly responsible for the shape of the egg (Pearl '09, and Curtis '14 b). Further this muscular activity is capable of regulatory changes. Pearl ('09) described the case of a pullet whose first eggs were much longer in proportion to their breadth than any other eggs produced at the Maine Station plant. There was a progressive regulatory change in the eggs of this bird until they finally became normal in shape.

5. The resection of one-fourth of the albumen secreting region and an end-to-end anastomosis of the remaining parts does not cause a permanent loss of function of the oviduct. Pearl and Surface ('08) described the results of such an operation. The bird began to lay four months after the operation. The eggs were only a little below the average size for the breed and appeared to have a normal proportion of albumen. There seems here also to be a compensatory action of the duct so that the amount of secretion is nearly normal. Whether there is an increase in the number of albumen glands or in the functional activity of those left is not known.

The experiments already described were confined to a study of the physiological processes of the oviduct itself. In all cases the functional integrity of the duct was preserved. During the course of the investigations into the physiology of reproduction, however, several operations have been performed where egg production has been prevented by the removal of a part or all of the oviduct, or by closing it at various levels. The purpose of the present paper is to show the results of such interferences upon, first, the growth of the ovary, second, the secondary sexual characters, third, the body metabolism, and fourth, the growth and ability to function of any remaining portion of the oviduct itself.

TECHNIQUE

The technique used in these operations was a simplified form of the technique of aseptic human surgery. A bird was given a subcutaneous injection of 1/200 grain atropine sulphate in 1 cc. of normal salt solution. Just sufficient ether was given to maintain complete insensibility (Pearl and Surface '09 b). On the bird was placed a clean muslin jacket which held the wings close to the body and covered most of the feathers. The feathers were picked off the field of operation and the skin painted with iodine.

The top of the operating table used is an especially constructed adjustable slat trough. This trough is adjusted so that when the back of the bird is at the bottom of the trough the ventral surface is even with the edges. The bird is secured to this table by means of strings attached to the legs and wings.

A sterile muslin screen attached to an iron hoop at one end of the table is stretched back over the bird. A hole is made in this screen in the proper place to expose the field of operation. This screen makes the bird's head accessible to the person giving the ether and yet shuts off this source of contamination from the field of operation. Sterile towels are placed above the screen so as to cover deeply the whole bird except the iodine

painted field of operation. Care is taken to have all instruments, sponges, and ligatures thoroughly sterilized.

The abdominal incision for operations on the oviduct is to the left of the middle line. The intestines are crowded to the right and covered with a piece of sterile vaselined silk and held with a retractor. The oviduct is thus exposed. After the operation the muscle incision is closed by several interrupted sutures with fine silk and the skin incision by continuous sutures with catgut. The closed incision is finally painted with collodion. In almost all the cases described the wound healed quickly by first intention and the stitches in the skin were removed about a week after the operation. In a few cases there was a slight granulation of the skin wound.

THE EFFECT OF INTERFERENCE WITH THE OVIDUCT UPON THE GROWTH OF THE OVARY, AND ITS FUNCTIONAL INTEGRITY

For many years it was an apparently generally accepted notion that the section or removal of a bird's oviduct caused degeneration of the ovary. Yarrell ('27) says that if a small portion of the oviduct is removed the ova do not afterward enlarge. According to Tegetmeier ('76) 'poulardes'² are fattened females rendered sterile when young pullets by cutting the oviduct, or by removing a portion of it. In regard to the effect of this operation he makes the following statement: "The development of the ovary or egg-producing organs is entirely prevented and the birds fatten rapidly attaining also a large size." Bland Sutton ('85) and Brandt ('89) also state on Yarrell's authority that removal of the oviduct causes the shrinking of the ovary.

It is well known from gynecological practice that in man the section or removal of portions of oviduct does not cause atrophy of the ovary. McIlroy ('12) showed that in rabbits, rats and guinea pigs removal of the ovary caused atrophy of the uterus, mammae, and external genitals, but removal of the uterus had no effect on the ovary. Sellheim ('07) has shown

² The term is still used in France, but is now in reality applied to any very fat female especially to a very fat pullet. They are often said to be sterilized females.

that in the domestic fowl also removal of the oviduct does not cause a permanent degeneration of the ovary. He states that the ovary at first shrinks but later is in the same condition as in unoperated birds. The yolks are discharged into the body cavity.

The results of twenty-nine operations on the oviduct are given in table 1. In twenty-four of these cases (all except 21 to 24 and 27) egg-laying was prevented. In fifteen (1 to 9 and 15 to 20) yolks could not enter the duct. In cases 15 to 20 practically the entire duct was removed when either in infantile or non-laying condition. The birds were autopsied at periods varying from ten days to nearly five years after the operation. They were in varying conditions of health and disease. Their ovaries presented the same range of variation shown in our large series of autopsy records on unoperated birds. Those of the normal healthy birds showed all stages from strictly non-laying to fully functional condition, while several of the birds with serious visceral lesions showed various stages of absorption of ovarian yolks. In no case is there the slightest evidence of atrophy of the ovary.

Several of the operations especially on the removal of the oviduct (cases 15 to 20) were performed before the sex organs had begun the rapid growth which precedes the first period of production in the pullet. In all of these cases the ovary continued to grow.

These results show that neither the ligation, section, nor entire removal of the oviduct causes degeneration or prevents the further growth of the ovary.

Sellheim ('07) states that after the removal of the oviduct the ovary at first shrinks. He believes this to be a result of the severe operation. Our experience shows that a bird is not in laying condition for some time after any serious abdominal operation involving prolonged anesthesia and considerable surgical shock. It seems quite possible that the 'shrinking' of the ovary noted is the return to a strictly non-laying condition due to a general physiological disturbance rather than the specific effect of the removal of the oviduct. This post-operative

TABLE 1

Showing the results of the ligation, section or removal of the oviduct

CASE NO.	OPERATION NO.	DATE OF OPERATION	AGE AT OPERATION	CONDITION OF OVIDUCT AT OPERATION	NATURE OF OPERATION	CAUSE OF DEATH	DATE OF AUTOPSY	BODY WEIGHT AT OPERATION: GRAMS	BODY WEIGHT AT AUTOPSY: GRAMS	LENGTH OF TIME AFTER OPERATION	CONDITION OF OVIDUCT AT AUTOPSY	REMARKS
1	84	Mar. 5 1909	9 mos.	laying	<i>Funnel mouth</i> closed with several interrupted sutures after insertion of artificial yolk (cork)	Internal bleeding from enlarged and ruptured spleen; some peritonitis	June 4 1909	2225	2572	447 3 mos.	Oviduct not very large but nearly; funnel still closed by sutures	Body fat; several small masses of hard yolk in body cavity
2	85	Mar. 5 1909	9 mos.	laying	<i>Funnel ligated</i> after the insertion of an artificial yolk (cork)	Killed for data	Mar. 18 1909	1070	2050	80 13 days	Oviduct in laying condition	All abdominal conditions normal
3	87	Apr. 14 1909	10 mos.	laying	<i>Funnel ligated</i> after the insertion of an artificial yolk (agar)	Internal bleeding from abdominal fat	Apr. 24 1909	1840	1455	385 10 days	Oviduct small; weight 0.8 grams	Utricles deposited on peritoneum covering viscera
4	95	Apr. 16 1909	10 mos.	laying	<i>Funnel ligated</i> after the insertion of an artificial yolk (one BB shot)	Killed for data	Apr. 29 1909	2070	1832	138 13 days	Oviduct in laying condition	A yolk walled off with peritoneum attached to funnel above ligature
5	97	Apr. 16 1909	9 mos.	laying	<i>Funnel ligated</i> after the insertion of an artificial yolk (clay marble)	Killed for data	Apr. 29 1909	1070	795	275 13 days	Oviduct resembled that of a bird which had not laid for 10 days to a month and without large yolks in ovary; glandular ridges deep	Bird in excellent condition; no infection; no adhesions

5	98	Apr. 16 12 mos. laying 1909	<i>Funnel</i> ligated after the insertion of an artificial yolk (marble)	Killed for data Apr. 29 1909	840	770	-70 13 days	Ovary in non-laying condition; a few small absorbing and a few small normal yolks	Oviduct in same condition as in case 5	Slight surface infection of wound; no internal infection; no adhesions
7	7	Jan. 16 8 mos. laying 1908	Removal of part of albumen portion of the oviduct and end-to-end anastomosis; later <i>funnel adhered to intestine some distance behind ovary</i> ; opening small and filled with a plug of hard yolk	Peritonitis Mar. 18 1910	2030	2030	2 yrs., 2 mos.	Ovary with three follicles; no egg with yolk	Oviduct in laying condition and some granular secretion was contained in the duct	Body cavity contained yolk masses and some semi-fluid yolk; bird had laid from March 17, 1908, to Oct. 6, 1909
8	96	Apr. 16 10 mos. laying 1909	The ligature which was placed around the funnel after the insertion of an artificial yolk was found at autopsy; untied but a large hyaline tumor was attached to one lip of the funnel and the other lip was closely pressed against the tumor. <i>Oviduct was therefore closed at the funnel mouth</i>	Killed for data May 10 1909	2180	2260	80 24 days	Four large yolks on ovary; wt. of largest 14.0	Oviduct in laying condition; wt. 54 grams	Wound healed. No infection and no adhesions; freshly broken yolk in body cavity

TABLE 1—Continued

CASE NO.	OPERATION NO.	DATE OF OPERATION	AGE AT OPERATION	CONDITION OF OVIDUCT AT OPERATION	NATURE OF OPERATION	CAUSE OF DEATH	DATE OF AUTOPSY	BODY WEIGHT AT OPERATION: GRAMS	BODY WEIGHT AT AUTOPSY: GRAMS	GAIN IN WEIGHT: GRAMS	LENGTH OF TIME AFTER OPERATION	CONDITION OF Ovary AT AUTOPSY	CONDITION OF OVIDUCT AT AUTOPSY	REMARKS
9	16	Oct. 23, 1905	8 mos.	infantile	Oviduct ligated at level of the shell gland	Killed by other birds sticking open body cavity at anus	Dec. 20, 1908	1500	2219	659	2 mos.	Three large yolks on ovary	I n. functional condition with two normal eggs in the duct just above the ligature; both these eggs had some shell	Body fat; wound completely healed; 5 normal and 2 yolkless eggs in body cavity; 3 of the normal eggs were surrounded with peritoneum and were in varying stages of absorption; three eggs had considerable shell; another half hard shelled egg was only partly surrounded by peritoneum and was not perceptibly absorbed; the other normal and the two yolkless eggs were without shell, were not surrounded by peritoneum and were not apparently absorbing

10	36 Nov. 13 64 mos. 1908	Infantile	Oviduct ligated at level of shell gland	Killed by other birds picking open body cavity	Mar. 7 1910	1446 After the viscera had been eaten out by other birds. Viscera of such a bird weigh more than 500 grams	1325 16 mos. (See note in preceding column)	Seven large normal yolks on ovary	Body fat; viscera eaten out by other birds except the ovary and a part of a yolk mass enclosed in peritoneum	
11	64 Feb. 16 9 mos. 1909	Infant	Resection of ischitis and end-to-end anastomosis; at autopsy it appeared that nothing had passed the anastomosis which was therefore virtually a ligature	Internal bleed- ing from ruptured spleen	Apr. 20 1909	2110	2 1/2 mos.	Large yolks on ovary but they were all hard	In laying condition; contained 7 egg masses, each surrounding a yolk and each hard; funnel enclosing and grown fast to hard yolk masses on ovary	Abdominal viscera a mass of adhesions
12	68 Feb. 18 9 mos. 1909	Infant	Reversal of portion of ischitis and abdominal portion: the piece was resected, turned end-for-end and anastomosed back into the duct; at autopsy duct was found shriveled and passage closed just below first anastomosis	Peritonitis	Mar. 11 1913	1295	4 yrs., 1 mo.	Not noted	Very large and filled with hardened egg masses; some of these were in the reversed portion but apparently none had passed the second ligature; shell gland and vagina were empty	Many yolk masses in body cavity surrounded by peritoneum and attached to varying peritoneal surfaces

TABLE 1—Continued

CASE NO.	OPERATION NO.	DATE OF OPERATION	AGE AT OPERATION	CONDITION OF OVIDUCT AT OPERATION	NATURE OF OPERATION	CAUSE OF DEATH	DATE OF AUTOPSY	BODY WEIGHT AT OPERATION, GRAMS	BODY WEIGHT AT AUTOPSY, GRAMS	GAIN IN WEIGHT, GRAMS	LENGTH OF TUBE AFTER OPERATION	CONDITION OF OVARY AT AUTOPSY	CONDITION OF OVIDUCT AT AUTOPSY	REMARKS
13	17	Oct. 29, 1908	5 mos.	infantile	Section of the duct, (also cut across between two ligatures)	Abdominal hernia and subsequent infection and peritonitis	Mar. 13, 1909	1390	2540	1150	5 mos.	Ten yolks on ovary; some hard; all abnormal	Both parts of the oviduct had enlarged to laying condition; no egg masses in duct but there was a lump of hard yolk just above the ligature, i. e., at end of first piece of the duct	Body fat; 5 yolks in body cavity walled off by peritoneum; peritonitis appeared due to infection from hernia
14	37	Nov. 13, 1908	13 7/8 mos.	infantile	Oviduct was cut across through the shell gland	Killed for date	June 21, 1909	1400	2294	894	7 1/2 mos.	Ovary in laying condition; 10 follicles varying in size from large to just visible ones; 3 large normal yolks; wt. largest 13.4 grams	Oviduct in laying condition; punctured 15 cm. above upper ligature; duct filled with egg masses made up of normal membrane and 1 covered absorbing egg with germ disk viable eggs; calcareous mass size of a common red cherry in shell gland; part of shell gland and vagina behind the cut were the size of these parts in a laying bird; vagina open into cloaca	Body fat; in body cavity were 9 empty egg membranes, 1 with a small amount of egg substance still unabsorbed and 1 perfectly fresh normal egg with germ disk viable eggs; calcareous mass size of a common red cherry in shell gland; part of bird seemed O.K.; no sign of peritonitis or putrefaction of egg masses

15	18	Oct. 30 1906	5 mos. infantile	Oviduct removed except the part of the vagina applied to the colon and the portion of the fused lips dorsal to the abdominal air sac	Killed for data	Aug. 6 1909	2286	1208	9 mos.	Ovary in normal condition; 6 yolks above 1 cm.; one quite large ruptured follicle	The small portions of the duct left at the operation had enlarged to the normal size of these parts in a laying hen; oviduct open into cloaca	Body very fat; no unabsorbed yolk in body cavity
16	34	Nov. 13 1908	5 mos. infantile	Oviduct removed with the exception of the part of the vagina closely applied to cloaca	Unknown	June 14 1909	1995	515	7 mos.	Ovary with 5 small absorbing yolks and some small normal ones; wt. with yolks 4.75 grams	Only the part of vagina attached to the colon present	Hard masses evidently yolk free in body cavity
47	35	Nov. 13 1908	5 mos. infantile	Oviduct removed with the exception of the part of the vagina closely applied to the cloaca	Killed for data	Aug. 24 1911	3789	2169	2 yrs., 9 mos.	Ovary with several large yolks; also several absorbing	Oviduct all gone except vagina in which was the size of the vagina in a laying hen	Body very fat; no yolks in body cavity
18	42	Nov. 20 1908	11 yrs. non-laying but adult	Oviduct removed except funnel and vagina	Stoppage of intestine due to adhesions	June 23 1909	2150	1936	7 mos.	Yolks on ovary hard	All gone except funnel portion; presence or size of vagina not recorded	Body cavity filled with yolk masses covered with peritoneum
19	45	Nov. 24 1908	6 mos. Beginning to enlarge for first laying period; about 15 cm. long	Oviduct removed except funnel, part of shell gland and vagina	Tuberculosis	July 14 1913	1800		4 yrs., 8 mos.	Many had partly absorbed yolks on ovary	Part of shell gland and vagina in condition of a bird which had lately been laying; size of funnel not recorded	Body cavity contained a large number of absorbed yolks enclosed in peritoneum
20	51	Dec. 3 1908	7 mos. infantile	Oviduct removed entire	Killed because she was sick	Feb. 23 1909	1070	1665	1 yr., 2 mos.	In healthy non-laying condition	Oviduct not found	Condition of other organs not recorded

TABLE 1—Continued

CASE NO.	OPERATION NO.	DATE OF OPERATION	AGE AT OPERATION	CONDITION OF OVIDUCT AT OPERATION	NATURE OF OPERATION	CAUSE OF DEATH	DATE OF AUTOPSY	BODY WEIGHT AT OPERATION, GRAMS	BODY WEIGHT AT AUTOPSY, GRAMS	GAIN IN WEIGHT, GRAMS	LENGTH OF TUBE AFTER OPERATION, CM.	CONDITION OF OVARY AT AUTOPSY	CONDITION OF OVIDUCT AT AUTOPSY	REMARKS
21	12	Oct. 22 1908	8 mos.	laying	Lumen of the abdominal region of duct diminished by taking a tuck in the muscular coat 14 cms. long	Killed for data	May 13 1909	1280	2125	845	63 mos.	In laying condition; 6 yolks on ovary; wt. of largest 15.5	In laying condition; no sign of the tuck; soft shelled egg in shell gland	Bird had been laying since Jan. 17, 1909
22	72	Feb. 10 1909	11 mos.	laying	Lumen of abdominal region of duct constricted by a tuck 4½ cms. long	Killed for data	May 14 1909	1530	1177	-353	3 mos.	In laying condition; 4 normal yolks above 1 cm.; wt. of largest 13.0 grams	Tuck was preserved in outer layer but it made no constriction in the lumen of the duct; egg at entrance of isthmus	Bird laid a normal egg Mar. 13, 1909
23	62	Feb. 16 1909	8 mos.	laying	Diminishing size of shell gland by removal of piece of the wall	Killed for data	May 13 1909		2140		3 mos.	In laying condition; 4 large yolks on ovary; wt. of largest 14.5 grams	Oviduct in laying condition; shell gland of normal shape although possibly a little small; silk still in tissue where cut	Bird has laid since Mar. 16, 1909; eggs about average size for breed
24	65	Feb. 17 1909	8 mos.	laying	Longitudinal incision of shell gland by interrupted sutures	Killed for data	May 14 1909	1600	1505	-95	3 mos.	In laying condition; 4 large normal yolks on ovary; wt. of largest 10.5	In normal laying condition; pieces of silk found in wall of shell gland; these did not appear to be tied; no appearance of tuck internally or externally	Bird had been laying since Mar. 13, 1909; eggs below average size for the breed but not low range of breed variation

25	75	Mar. 4 0 mos. 1909	laying; egg left in shell; gland	Ventral ligament cut where it is a round mass of muscle on ventral side of shell gland; funnel also ligated; Removal of en- tire ventral liga- ment	Bird was acci- dentally sold in summer 1910						Laid the egg which was in shell gland at the time of operation on the day following; did not lay again	
29	109	Oct. 29 3 mos. 1909	infan- tile	Removal of en- tire ventral liga- ment	Killed because of peritonitis	June 4 1910	1400	1375	886 3/4 mos.	Several large ab- sorbing yolks on ovary	Ventral ligament lacking; duct size of a duct of a bird which had laid 10 days previously; vagina open to cloaca	Bird had never laid but had ovi- lated into body cavity
27	112	Nov. 4 5 1/2 mos. 1909	infan- tile	Piece of the ven- tral ligament 3 cm. long removed	Killed because of peritonitis	March 24 1910	1620	2063	473 1/2 mos.		Ventral ligament lacking through- out the length of the isthmus but normal at upper end and there was a hard mass of muscle along the ventral side of shell gland	Had laid several normal eggs
28	113	Nov. 4 7 mos. 1909	infan- tile	Piece of the ven- tral ligament 1 cm. long removed	Killed	Dec. 7 1910	1500	1939	339 13 mos.	In non-laying condition	Not in laying condition; ventral ligament at head of shell gland wanting	Had not laid; no evidence that the bird had ever been in laying condi- tion; viscera nor- mal
29	119	Nov. 18 4 mos. 1909	infan- tile	Piece of the ven- tral ligament 0.7 cm. long removed	Digestive dis- orders	March 11 1910	435	429	-6 4 mos.	In non-laying condition	Not in laying condition; a part of the ventral liga- ment along the shell gland want- ing	Had not laid and there is no evidence that the bird had ever been in laying condition

'shrinking' no doubt accounts for the belief of the early workers that extirpation of the oviduct caused degeneration of the ovary. Our experiments on the removal of the oviduct do not bear upon this point since at the time the ducts were removed (cases 15 to 20) the sex organs were in infantile or non-laying condition and they were not examined for at least seven months after the operation. Cases 2 and 5, however, show that the ovary may be in full functional condition 13 days after the funnel is ligated.

THE EFFECT OF INTERFERENCE WITH THE OVIDUCT UPON THE SECONDARY SEXUAL CHARACTERS

The early workers Yarrell ('27), Bland Sutton ('85), and Brandt ('89) state that the removal of the oviducts is followed by the assumption of male secondary sexual characters by the female. The belief of the early workers that removal of the oviduct caused the degeneration of the ovary no doubt led them to misinterpret the normal growth of the comb, wattles, and spurs, as the assumption of male characters.

None of the birds from which we removed or otherwise interfered with the oviduct showed any tendency to assume male characters. At the time of autopsy they were as female in all secondary sexual characters as normal birds. This result also agrees with those of Sellheim ('07). He summarized the results of his experiments as follows:

Damit ist für die Henne der Beweis geliefert, dass die Entfernung des Legdarmes keine schädliche Wirkung auf die Funktion des Eierstockes ausübt. Dass die Legdarmresektion in ihrer Wirkung einer Kastration gleich setzen sei und dass die sekundären Geschlechtscharaktere, Bartläppchen, Kämme, Sporen, Gefieder, Beckenform, Stimme, benchmen gegen das andere Geschlecht nach der Exstirpation des Legdarmes sich ändern, ist reines Phantasiestück.

THE EFFECT OF THE LIGATION OR REMOVAL OF THE FUNNEL UPON OVULATION

Coste ('74) describes the infundibulum embracing the ovarian yolk in its follicle directly before the time for ovulation. The same phenomenon has since been observed many times by other workers, including the authors. So far as is known the in-

fundibulum has never been observed³ in the act of swallowing a free yolk. Further, the work of Patterson ('10) and Bartelmez ('12) shows that normally the follicular orientation is preserved in the oviduct. These considerations make it certain that normally the yolk is ovulated into the enclosing funnel.

Coste believed that the pressure of the funnel upon the follicle was the probable cause of ovulation and Patterson ('10) apparently accepts this view. The walls of the funnel are muscular and at the time it embraces the follicle it is in active peristalsis. That the pressure is sufficient to cause or at least materially to aid ovulation is a natural inference.

³ That yolks set free in the body cavity may subsequently enter an oviduct seemed the most reasonable explanation for the conditions observed in two birds (no. 17K and 397K) autopsied at this Laboratory. Both these birds were pure bred Barred Plymouth Rock hens a little over one year old and each had laid a number of eggs. Bird no. 17K had not laid for four months and bird 397K had not laid for one month. At autopsy the sex organs on the left side of the body of each bird were in practically non-laying condition. In neither bird was there any right ovary. Each bird had what appeared to be a right oviduct filled with large egg concretions. These concretions were exactly similar to such masses often found in abnormal conditions of the left oviduct. They appeared to consist of concentric layers of hardened albumen surrounding hardened yolks. In both birds the upper end of the tube was greatly distended. Its walls were stretched thin as is a left oviduct containing large egg masses. Smooth muscle fibers were visible in the walls. Each bird showed considerable peritonitis and there were adhesions between the walls of the tube (oviduct) and intestine. The funnel mouth could not be distinguished. The lips had apparently adhered together; (this is often true in the case of egg concretions in the left oviduct). The tube continued for several centimeters behind the caudal end of the masses. It ended blindly near the cloaca into which it did not open. There was no differentiated shell gland. In Bird no. 397K the tube had ligaments very much like normal oviduct ligaments. In Bird no. 17K the ligaments were not exactly like normal oviduct ligaments but the tube was held in a fold of peritoneum. There seemed no doubt that these egg masses were egg concretions formed in the rudimentary right oviducts and little doubt that the centre of these masses were yolks. If this was true the yolks must have been ovulated from the left ovary and have passed across the body cavity (behind the gizzard in Bird 17K and by this path or through a hole which was found in the mesentery in Bird 397K) and there have been picked up by the right oviduct. It should possibly be added that other large right oviducts have been observed in the routine autopsy work. One case (Bird no. 276) had both right and left oviducts in nearly functional condition. The typical parts were differentiated in the right duct and it was open into the cloaca. In this case also there was no right ovary.

Bartelmez ('12) has shown that in the pigeon continued yolk formation increases the pressure within the follicle so that "the egg bulges out when the rupture begins" and "the egg is over a millimeter in diameter greater after ovulation than the whole follicle was before." He and other workers recognize in this increase in internal pressure an important factor in the rupture of the follicle.

That the pressure of the infundibulum is not necessary for the rupture of the follicle is proven by the experiments of Sellheim ('07), which show that after the oviduct is removed ovulation takes place into the body cavity. This result is confirmed by the present investigation which shows that ovulation into the body cavity occurs when the funnel mouth is closed by sewing or ligating the ostium (cases 1, 4, 7 and 8), or by removal of the duct (cases 15, 16, 18 and 19). In cases 4, 8, 15 and 17 the birds were killed during a normal period of egg production and (with the exception of case 17) showed evidence of recent ovulation into the body cavity. In these birds none of the yolks remaining in the ovary were apparently larger than yolks found in the ovaries of normal laying birds of the same breed. Quantitative data are not as yet available on this point, but the observations indicate that absence of pressure exerted by the funnel does not perceptibly delay ovulation. This suggests not only that internal pressure is a sufficient cause for rupture of the follicle, but also that it may be the most important factor in causing such rupture in the case of normal ovulation into the oviduct.

THE EFFECT OF PREVENTION OF EGG-LAYING ON BODY METABOLISM

It has already been shown that if the oviduct is removed or if the mouth of the duct is permanently closed the ovary passes through normal periods of egg production and the yolks are ovulated into the body cavity. Cases 9, 10, 12, 13 and 14 show that when it is possible for a yolk to enter but not to leave the duct, the duct may be filled with egg masses and ovu-

lation then takes place into the body cavity or if the duct be stopped at the level of the shell gland normal eggs may be formed and passed back up the duct into the body cavity. The fate of these yolks and eggs and their effect on body metabolism is of some interest.

At the height of a period of egg production a bird is consuming a large amount of food materials which she is elaborating into eggs. In the cases cited she is discharging these products into her own body. Is she able to resorb these eggs and if so by what process is it accomplished? And are the resorbed eggs utilized in the body metabolism?

There are in all fourteen cases where the bird at autopsy showed that she had been either ovulating (cases 1, 4, 7, 8, 10, 12, 13, 15, 16, 18, 19 and 26) or backing fully formed eggs (cases 9 and 14) into the body cavity. Four of these fourteen (cases 1, 7, 12 and 26) died from peritonitis which may have been caused by inability to absorb the yolks in the body cavity. The other ten birds, or 71 per cent, were able to resorb the yolks or eggs. Moreover, in none of the four cases of peritonitis were we certain that it was due to the presence of the yolks although this seemed the most probable cause.

In addition to the fourteen cases cited in the preceding paragraph there are in the archives of the Laboratory autopsy records of other birds which at the time of death were ovulating into the body cavity and resorbing the yolks. Two methods of resorption are observed. First, absorption directly through the general peritoneal surface, and second, walling off of the yolks or eggs by peritoneum and subsequent absorption. In cases 1, 7, 8, 14, 15⁴ and 16 absorption was by the first method. None of these cases present such clear evidence of rapid absorption of several yolks as some cases met with in the routine autopsy work. Descriptions of two such cases follow.

Bird no. 1406 laid but seven eggs during her life. The last of these was laid August 22 of her pullet year. She was kept

⁴ In this case the yolk had been entirely absorbed but the follicle on the ovary was large and it therefore seemed impossible that sufficient time had elapsed for absorption by the second method.

until February 6 of her second year when she was killed for data. She was twenty-two months old and was a large and very fat bird. In the uterus was a small membrane-shelled egg weighing only 23 grams. The yolk of this egg weighed only 4.87 grams. The oviduct was normal except that the opening of the funnel seemed small. In the ovary were four yolks above one centimeter and six between a centimeter and a millimeter in diameter. The ovarian yolks were a normal series of maturing yolks, the largest of which was apparently too large to enter the oviduct. The ovary also contained four large follicles. In the body cavity was a creamy oily fluid. This was apparently yolk mixed with serum. There was no appearance of peritonitis; in fact the bird was apparently in perfect health.

Bird no. 1375 laid reasonably well during her first two laying years producing 141 eggs the first year and 117 the second but after the second adult molt she laid but one egg (February 14, 1914). On March 7, 1914, she was killed for data. She was thirty-five months old and was very fat. On opening the sheet of fat enclosing the viscera they were seen to be covered with thick fresh egg yolk. The peritoneum was perfectly normal. The oviduct was large and normal in appearance. The finger could easily be inserted into the funnel mouth which was apparently large enough to admit a normal mature yolk. The duct was open throughout. The glandular ridges of the albumen secreting and isthmus regions were expanded and whitish, as if full of secretion. There was a little albumen between the ridges in the albumen secreting portion. The funnel hung rather loosely from its ligamentary attachments. When the body cavity was opened the *ostium* was situated at some distance behind the largest yolk. The intestines were still capable of normal peristalsis and slight peristaltic movements could be induced in the oviduct. Quantitative data are lacking but the response of the duct seemed less vigorous than the normal response of the oviducts of laying birds when stimulated immediately after death. In the ovary was a large empty follicle apparently just discharged and five others ranging gradually in size from this to one about 1 mm. in diameter. There were also

very many which were just distinguishable. The ovary also contained a normal series of maturing yolks. Four of these were above 1 cm. in diameter. The largest was apparently mature.

These two birds then had normal ovaries and at the time of death were maturing and ovulating yolks at a rate comparable to that shown by birds at the height of a normal period of egg production. They also possessed oviducts which were apparently able to secrete the normal enclosing envelopes. The fact that the yolks did not enter the duct and become the yolks of normal eggs was probably due, in the first case, to the fact that the funnel mouth was too small to admit a full sized yolk, and in the second to a lowered state of tonus in the muscles of the oviduct and oviduct ligaments. They represent a type of non-production previously discussed by one of the authors (Pearl '12) where sterility is due to 'somatic' (physiological) rather than 'gametic' causes. From the point of view of the present investigation they show that a bird may ovulate into the body cavity and resorb the yolks from the general peritoneal surface at an astonishingly rapid rate without causing any apparent disturbance of normal metabolism.

In the second type of absorption in the body cavity the yolks or eggs are enclosed in separate sheets of peritoneum by which they are attached to the adjacent peritoneal surface. This type of absorption has been several times observed in routine autopsy work as well as in cases 4, 10, 12, 13, 18 and 19. Case 9 shows all the steps in the process.

This bird's oviduct had been ligated at the level of the caudal end of the shell gland before it has enlarged for its first period of egg production. The sex organs developed normally. The duct received the yolks and formed normal eggs which were discharged back through the funnel into the body cavity. At the time of autopsy the body cavity contained five normal and two yolkless eggs, and there were also two normal soft shelled eggs in the oviduct just above the ligature. The condition and position of the eggs in the body cavity were as follows:

- (1) A collapsed shell, slightly calcareous, containing a small

amount of mixed albumen and yolk was surrounded by peritoneum by which it was firmly attached to the abdominal fat and the right side of the colon at the posterior end of the body cavity. (2) A partly collapsed soft shelled egg, surrounded by peritoneum which attached it to the peritoneum covering the left kidney, lay between the left kidney and the oviduct just behind the ovary. (3) A soft shelled egg, surrounded by a very thin sheet of peritoneum, was attached to the right side of the intestinal mesentery just opposite the ovary. (4) A normal hard shelled egg occupied the posterior end of the body cavity to the left of the intestine. A part of the shell was covered with peritoneum. (5) A soft shelled egg not surrounded by peritoneum lay just behind 3, into the side of which it was indented. (6) Two yolkless membrane shelled eggs were free in the body cavity among the convolutions of the oviduct.

This bird was clearly discharging eggs into the body cavity at a rapid rate. These eggs were evidently moved about in the body cavity by the movements of the intestine and oviduct until they were pushed into some portion of the cavity where they remained quiet long enough for the local peritoneal surface to throw out a surrounding envelope of peritoneum. The four eggs attached or in the process of being attached were at the four corners of the abdominal cavity and were therefore in positions least likely to be affected by the movements of digestion and egg formation.

It sometimes happens that yolks become attached to the peritoneum forming the intestinal mesentery or the oviduct ligaments. Apparently if an egg or a yolk free in the body cavity is allowed to remain quietly in one position the adjacent peritoneum throws an enclosing membrane around it. If, on the other hand, it is kept in motion especially if it becomes broken and spread out over the general peritoneal surface it is not enclosed by peritoneum but is absorbed directly.

Whether the eggs are absorbed directly or after they are enclosed by peritoneum must depend partly on the rapidity with which they enter the body cavity, partly upon the strength

of peristaltic movements of the intestines and oviduct, together with the chance of being lodged in some undisturbed position, and partly also upon the general physiological tone of the individual and the consequent power of absorption from the general peritoneal surface.

From his study of "the effect of variations in body position and in gastrointestinal activity upon the degree and extent of diffusion of benign and toxic substances when introduced intraperitoneally" and of "the influence of the degree of diffusion upon the absorption of toxins" Yates ('08) concludes that:

1. Intraperitoneal diffusion results from the operation of purely physical forces abetted by physiological activities.
2. The greatest attainable restriction of this diffusion must result from the opposition of controllable physical force to the paths of greatest extension and the inhibition of physiological activities.
3. Under similar conditions the rate of absorption varies directly with the extent of diffusion.

Our observations on the absorption of eggs discharged into the body cavity are in general agreement with Yates' experimental results.

When a bird is laying she is transforming a large amount of material into egg substance. Gerhartz ('14) has recently made accurate measurements of the energy contained in the egg and of that utilized in the digestion and absorption of the extra food and in converting body substance into egg substance. With the laying of the egg all this energy is lost to the individual. When the egg is deposited in the body cavity and resorbed the energy contained in the egg, minus the energy required for the resorption, is retained by the individual. When egg formation is stopped at ovulation, the ovulated yolk being resorbed from the body cavity, not only is the energy contained in the yolk saved to the individual but also the energy which would have been contained in the egg envelopes and that which would have been utilized in their elaboration. If the energy consumed in the resorption of yolks or eggs from the body cavity is less than the energy contained in fully formed eggs,

and if birds which are ovulating or extruding eggs into the body cavity continue to digest and absorb the same amount of food as birds in similar periods of reproduction, the birds which are prevented from laying eggs will accumulate reserve energy.

The experiments so far as they go suggest that birds which resorb yolks or eggs from their body cavity may utilize this resorbed material (and the material not used to complete the eggs) in the production of body fat. There are six cases (1, 9, 10, 13, 14 and 15) which show evidence on this point. These birds were all killed for data while in good health except cases 1 and 13. Case 1 died as the result of the rupture of an enlarged spleen. Case 13 died from the infection of the body cavity through an abdominal hernia. All these birds had made large gains in body weight since the operation and all were *very fat* at the time of autopsy. The autopsy records quoted on page 412 show that the unoperated birds which were ovulating into the body cavity and resorbing the yolks were also very fat. The other birds which had ovulated into the body cavity were either killed too soon after the operation to have resorbed many yolks (cases 4 and 8) or died from diseases which might have prevented the accumulation of body fat (cases 7, 12, 16, 18, 19 and 26). Some of these cases also had made substantial gains in body weight but they did not appear to be abnormally fat at the time of autopsy.

Too much weight cannot be placed on the evidence from these experiments, since most of the birds were not fully grown at the time of operation and a part of the gain in body weight is due to normal growth. Further the cases are too few to determine statistically that these birds had accumulated more body fat than laying birds of the same age. This, however, appears to be the case.

THE EFFECT OF OPERATIONS ON THE OVIDUCT UPON ITS GROWTH
AND ABILITY TO FUNCTION*1. Diminishing the caliber of the duct*

Four operations were performed to test the effect of diminishing the diameter of the oviduct. In cases 21 and 22 the size of the albumen secreting region of the duct was decreased for several centimeters by taking a tuck in the wall. In case 23 the size of the shell gland was diminished by cutting out a piece of the wall. In case 24 the shell gland was made smaller by inverting a longitudinal portion of the wall. All these birds laid normal eggs. In size and shape these eggs were within the range of variation shown by the eggs of unoperated birds of the same breed. At autopsy the stitches which had made the tucks had either pulled out (cases 21 and 24) or the tuck was preserved only as a ridge on the muscular wall (case 22). In case 23 where a piece of the wall of the shell gland had been removed this part was of normal shape. It looked a little small. This bird, however, had laid eggs fully up to the average size for her breed.

The operations attempted did not perceptibly influence the size or shape of the egg. Many well controlled experiments would be necessary to demonstrate that it is impossible to alter the size and shape of the egg by diminishing the diameter of the tube. In three of the four operations the size of the tube was not permanently diminished because the stitches did not hold.

2. Closing the duct at various levels

When the funnel is closed by sewing the lips, or by ligating the *ostium*, yolks cannot enter the duct. Yet in such cases the duct passes through the normal phases of reproduction. In cases 1 to 6 the oviducts were in laying condition at the time the ostium was closed, either by sewing the lips (case 1) or ligating the funnel mouth (cases 2 to 6). At the time of autopsy thirteen days to two months later the oviduct of each bird was in a condition of reproduction corresponding to the condition of the ovary. Two of these birds had ovulated into the body

cavity but there is no evidence that any secretion had been formed by the duct. Closing the mouth of the duct does not prevent the duct from passing through the cyclic changes which are coördinated with the cyclic changes in the ovary. The duct glands, however, do not ordinarily discharge their secretion except under the stimulation of the presence of the yolk. This is in accord with the result of Pearl and Surface ('09 a) on the shell gland.

Cases 9 to 14 show the effect of closing the duct at other levels. The duct was closed by a ligature in cases 9 and 10; by an operation which resulted in the permanent shrinking of a small portion of the tube in cases 11 and 12; and by the section of the duct between two ligatures in cases 13 and 14. In all these cases the oviduct was in full functional condition at autopsy. In every case the duct on both sides of the ligature or cut had enlarged to the size of an oviduct in a laying bird. Yolks had entered these ducts and had stimulated the secretion of the duct as far as the point where the passage was closed. In no case was there any secretion posterior to the ligature. That is, the whole duct enlarged but it was able to function only under the stimulation of the advancing egg.

There is possibly a difference in the final response of the duct according to the level at which the passage is stopped. Where the duct was closed through the shell gland (cases 9 and 14), membrane covered or soft shelled eggs were formed and passed back up the duct into the body cavity. In cases 11 and 12 the ducts were closed at the isthmus and in each case the duct above this point was filled with masses of secretion which surrounded yolks but they did not pass back into the body cavity. In case 12 there were yolk masses in the body cavity surrounded by peritoneum but these did not appear to contain albumen. It is possible that an egg cannot stimulate sufficient antiperistalsis to cause its extrusion from the duct until it has become somewhat firm by the formation of the egg membrane. The number of cases, however, are too few, and the possibility of the operation of other causes too great, to place much weight on this interpretation.

3. Removal of the larger part of the duct

In cases 15 to 20 the oviduct was almost entirely removed. In most cases a portion of the funnel and a portion of the vagina were left. All these ducts were far from laying condition at the time of the operation. Case 17 was just beginning to enlarge for its first laying period. It was only 15 cm. long. The others were in infantile or non-laying condition. An examination of the autopsy records made from seven months to four years and eight months after the operation shows, first, that whatever portion had been left at the time of operation had remained, and second, that at the time of autopsy it exhibited the condition which one would expect to find for that portion of the entire oviduct in a bird with a similar condition of the ovary. This shows that the removal of the greater portion of the oviduct does not cause the atrophy of any remaining portion. In fact any part of the duct not removed apparently passes through growth and cyclic changes associated with the periods of ovarian yolk production exactly the same as though the duct were intact. This result was obtained also by Sellheim ('07).

4. Section or removal of all or a part of the ventral ligament

The peritoneal sheet which enfolds the oviduct projects as a fold along the ventral margin of the duct. In this fold of peritoneum there is developed a considerable amount of smooth muscle. This peritoneal fold with its contained muscle has been described by one of the authors (Curtis '10) as the ventral ligament of the oviduct. In a laying bird the extreme ventral margin of this ligament is a solid muscular cord, three to six millimeters in diameter. It becomes heavier toward its caudal end.

Muscle bundles pass from it to the duct where they are continuous with fibers in the longitudinal muscular layer of the wall. Other bundles pass to the colon and cloaca. Large bundles also cross the uterus and attach to the body wall muscles in the anal region. The forward end of the ligament suspends the backward prolongation of the funnel lips.

The anatomical relations of the ligaments and the distribution of the muscle fibers suggest that it aids in the peristaltic movements of the duct and may be of great importance both in the reception of the yolk by the duct and in the extrusion of the mature eggs. Cases 25 to 29 were planned to test the part played by the ventral ligament. In case 25 it was cut and in cases 26 to 29 all or a part of it was removed.

Case 25 was a laying bird with an egg in the shell gland at the time of operation. The round cord of muscle on the ventral side of the head of the shell gland was cut. On the next day the bird laid the egg which was in the shell gland at the time of operation. She never laid again. The anatomical effect of the operation is not known since the bird was accidentally sold more than a year after the operation.* The operation did not prevent the laying of the egg already formed but apparently prevented the laying of any others.

Case 26 was a three months old pullet with the oviduct in infantile condition at the time of operation. The entire ventral ligament was removed. The bird never laid. At autopsy eight and one-fourth months after the operation it was found that she had ovulated into the body cavity. The oviduct was in the condition corresponding to the condition of the ovary but there was no sign that a yolk had ever entered the duct.

At the time of operation case 27 was a five and one-half months pullet with oviduct still in infantile condition. Three-fourths of a centimeter of the ventral ligament in the isthmus region was removed. The bird developed normally and laid several normal eggs. At autopsy four and three-fourths months after the operation the ventral ligament was found in normal condition at the upper end. The funnel attachments were normal. The ligament was lacking entirely in the isthmus region. On the ventral and lateral wall of the uterus closely adhered to the wall was a thick mass of muscle which extended across the uterus and sent fibers both dorsally and ventrally along the uterus wall. Also there was a bundle of fibers which passed along the ventral margin of the wall and on to the vagina. The distribution of these fiber bundles was quite different from

the distribution of those of a normal ventral ligament but their contraction would be very effectual in diminishing the size of the uterus and therefore in expelling the egg.

Cases 28 and 29 were also young pullets from which portions of the ventral ligament were removed before the first enlargement of the reproductive apparatus. Neither of these birds ever laid. They were autopsied when in strictly non-laying condition. The ventral ligament was lacking at the head of the uterus. If they had ever passed through periods of reproductive activity in which they ovulated into the body cavity, they had completely resorbed all the yolks. These cases do not therefore contribute to our knowledge of the function of the ventral ligament since there was no proof that either bird had been in laying condition. They, however, do agree with the results from the other cases that if any portion of the ligament is removed it is not replaced but all the remaining portions develop. In case 27 described in the preceding paragraph there was, however, a compensating development of muscle fibers in the uterus wall so that expulsion of the egg was possible.

These experiments on the ventral ligament point strongly to the conclusion that the action of the forward part of the ligament is of great importance for the ovulation of the yolk into the duct either by holding the funnel in position or by the movements induced in the funnel. Since an egg cannot be extruded unless it is formed, only two of these cases (25 and 27) have any direct bearing on the question of the function of the ligament in egg laying. Each of these birds could extrude eggs after the operation on the ligament. In case 25 the ligament was simply cut across. It is quite possible that this may not have greatly interfered with the normal muscular action of the muscle bundles in the uterus region. In case 27 there was an unusual development of the muscle fibers on the wall of the uterus. The contraction of these fibers would have much the same effect as the contraction of the muscle bundles from the ligament. This suggests that the muscle bundles contained in and arising from the ventral ligaments are probably an important part of the normal apparatus which expels the egg.

SUMMARY

1. Neither the ligation, section, nor entire removal of the oviduct causes the degeneration or prevents the further growth of the ovary.

2. The pressure of the enclosing funnel is evidently not necessary to ovulation since yolks are ovulated into the body cavity after the *ostium* is sewed or ligated or after the entire duct is removed.

3. Internal pressure due to continued yolk formation is probably the most important factor in the normal rupture of the follicle, since closing the funnel or removing the duct apparently does not greatly delay ovulation.

4. There are cases of unoperated birds with normally functioning ovaries, and oviducts apparently capable of functioning, which do not produce eggs because of some anatomical or physiological condition of the mouth of the oviduct which prevents the entrance of the yolk.

5. The fate of yolks or eggs set free in the body cavity depends apparently upon the physiological vigor of the bird. First, they may cause serious metabolic disturbances which result in the death of the bird; second, they may be absorbed rapidly from the general peritoneal surface; or third, they may be walled off by peritoneum and then absorbed.

6. The material from the resorbed yolks or eggs is apparently utilized in body metabolism since all such birds which were in good health at the time of autopsy were very fat.

7. The removal of the greater portion of an oviduct does not cause the atrophy of any remaining portion.

8. The whole or any remaining part of an oviduct sewed at the funnel, ligated at any level, or with parts removed, passes through growth and cyclic changes coordinated with changes in the ovary exactly as an unoperated duct.

9. The stimulation of the advancing egg is necessary for the discharge of the secretion of the duct, since a duct closed at any level functions only to the point where the passage is interrupted.

10. When any portion of the ventral ligament is removed it is not replaced but all remaining portions develop.
11. The forward portion of the ventral ligament is necessary for the reception of the yolk by the funnel.
12. The muscle bundles which arise from the muscular cord in the ventral ligament along the uterus are probably an important part of the normal apparatus which expels the egg.

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NORMAL PERIODIC REORGANIZATION PROCESS WITHOUT CELL FUSION IN PARAMAECIUM

LORANDE LOSS WOODRUFF AND RHODA ERDMANN

Osborn Zoological Laboratory, Yale University

SIXTY-SIX FIGURES (FOUR DOUBLE COLORED PLATES)

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I. INTRODUCTION

The conception of the Protozoa as primitive animals has led to innumerable studies on these forms—morphologists and physiologists alike being animated with the idea that here many of the riddles of life propounded by the so-called higher animals would be presented in a simple form and so be more readily solved. While this view of the Protozoa is undoubtedly true

in a broad sense, recent studies on these organisms have only served to emphasize that size and simplicity do not necessarily go hand in hand and that a unicellular or non-cellular animal with all its varied life processes performed within the confines of a protoplasmic unit presents difficulties which differ in kind rather than in degree from those encountered in the metazoa. No better instance of the truth of this could be presented than that afforded by studies on the life history of the Infusoria. Dujardin's ideas of simplicity—brought forward to combat Ehrenberg's mistaken interpretations of the complexities which he observed—gradually were displaced by the revelations of Balbiani, Bütschli, Engelmann, Maupas, Hertwig and others on the complicated phenomena of conjugation, while the results of more recent work on conjugation from the standpoint of dynamics and heredity have only served to emphasize the intricacies of the infusorian life processes. The present study fully describes still another complex nuclear phenomenon¹ which we have discovered in the life of *Paramaecium aurelia* and which we believe affords the key to certain apparent contradictions in recent results on the life history of the Infusoria.

The problems of protoplasmic senescence and the significance of fertilization have afforded the stimulus for a long series of investigations on the life history of Infusoria since, more than three-quarters of a century ago, the leading students of microscopic organisms, Ehrenberg and Dujardin, theorized on the potential immortality of these forms. It remained, however, for Bütschli ('76) and Engelmann ('76) to attack the problem experimentally, in the light of zoölogical advance in the intervening years, and to reach the conclusion that continued reproduction by division results in degeneration and death; while the classic studies by Maupas ('89) on the life history of a number of different species of Infusoria afforded such a wealth of evidence pointing in the same direction that conjugation as a sine

¹ We have already given brief outlines of this process in the *Proc. of the Society for Exper. Biol. and Med.*, vol. 11, no. 3, February 18, 1914, and in *Biol. Cent.*, Bd. 34, 1914. In the latter several errors are present owing to the war preventing a revision of proof.

qua non for the life of the Infusoria seemed to be placed upon a firm empirical basis.

A series of careful investigations by R. Hertwig ('00-'04) and Calkins ('02-'04) confirmed Maupas' general conclusion that Infusoria after a more or less definite number of divisions degenerate and finally die if conjugation is prevented. Further, the latter author found that artificial stimuli of different kinds may be substituted with success for conjugation since by the opportune use of artificial stimulation he was able to prolong the life of one culture of *Paramecium caudatum* to the 742d generation. This significant discovery that the death of infusorian cultures, bred on a more or less constant medium of hay infusion, may be temporarily deferred by artificial means was corroborated by Woodruff ('05).

Enriques ('03) studied the same general problem and reached the conclusion that the degeneration and death observed by Calkins and others was due to the presence of bacterial poisons in view of the fact that he succeeded in breeding *Glaucoma scintillans* for 683 generations without signs of degeneration when this factor was eliminated. Although Enriques' interpretation of the cause of degeneration in Calkins' cultures is open to question, the significant fact remains that he kept his animals nearly twice as long as did Maupas or Calkins without conjugation or artificial stimulation, thus suggesting that degeneration is not inevitable, and that if suitable conditions are supplied reproduction by division can proceed indefinitely.

The problem was attacked from another point of view by Woodruff (1907 to date) who investigated the possibility that the degeneration observed in the previous investigations was induced by too great uniformity in the conditions of culture, or by the culture medium being deficient in something essential for the continued well-being of the organisms. A race of *Paramecium aurelia* (I) was isolated and bred on infusions of various materials found in the natural environment of the animal, while a sub-culture was subjected to the relatively constant hay infusion culture conditions employed by Calkins ('04). The result was that the cells bred in the constant hay infusion medium died

out after a typical Calkins cycle, while those bred on the 'varied environment' medium did not pass through periods of marked physiological depression or show morphological changes which could be interpreted as abnormal. This race is still (June, 1914) in a normal condition, having attained over 4500 generations without conjugation or the use of artificial stimuli.

The success with the varied culture medium naturally led to the question whether the longevity of *Paramecium* on a varied environment is dependent upon intrinsic stimuli from the frequent changes of the medium, or whether a constant medium of hay infusion is unfavorable because it lacks some elements which are essential for the continued existence of the organism. Accordingly, Woodruff and Baitzell ('11 a) bred a sub-culture of this race for a period of nine months on a constant culture medium of beef extract. The continued health of the organisms on this constant medium throughout the experiment, which was continued sufficiently long to include a Calkins cycle if such was inherent, indicated that it is the composition of the medium rather than the changes in the medium which is conducive to the unlimited development of this race without the necessity of conjugation or artificial stimulation.

From a study of various species of hypotrichous Infusoria, as well as his main culture of *Paramecium aurelia*, Woodruff found that minor periodic rises and falls of the division rate occur from which recovery is autonomous. He termed these fluctuations 'rhythms' and contrasted them with the so-called cycle, which comprises a varying number of rhythms and, according to Maupas and Calkins, ends in the death of the race if conjugation or artificial stimulation is not resorted to. The problem of the rhythms was studied intensively by Woodruff and Baitzell ('11 b) who showed that when *Paramecium aurelia* is subjected to the most constant environmental conditions it is impossible to eliminate the rhythm and thus resolve the graph of the multiplication rate into an approximately straight line. Accordingly there are inherent rhythmical changes in the phenomena of the cell which produce slight changes of the division rate. The same result was reached by Woodruff and Baitzell

('11 c) from a study of the temperature coefficient of the rate of reproduction of this culture which showed that the rate of cell division of *Paramecium* is influenced by the temperature at a velocity similar to that for a chemical reaction—except when the rhythms interfere.

The results from the study of this pedigreed race of *Paramecium aurelia* have led Woodruff to conclude that this organism, when subjected to suitable culture conditions, has the power of unlimited reproduction by division without conjugation or artificial stimulation—the only necessary variations in the rate of reproduction being the normal minor periodic rise and fall of the division rate (rhythm), due to some unknown factor in cell phenomena, from which recovery is autonomous.

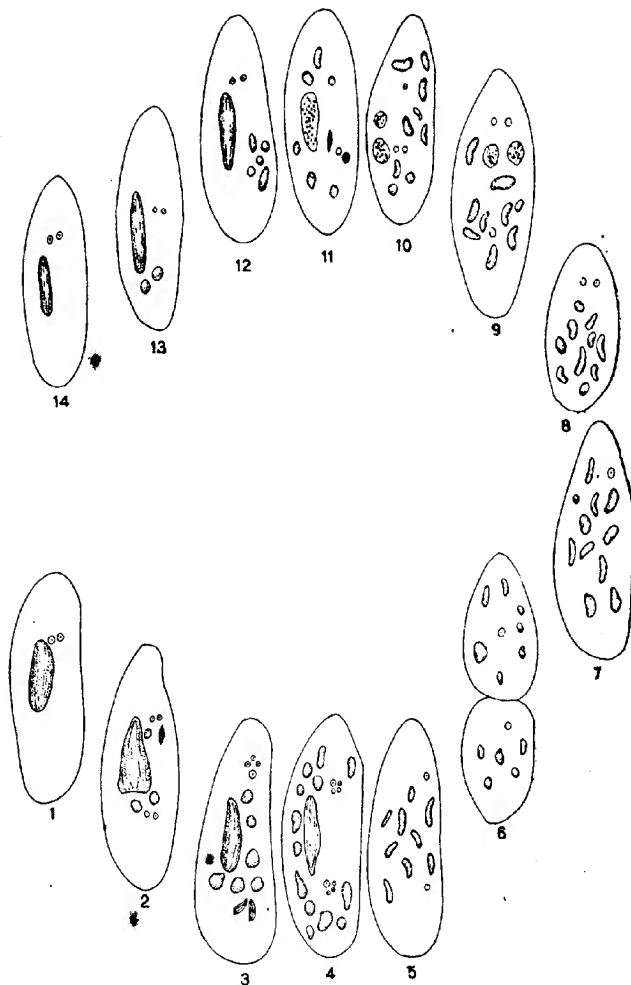
Calkins and Gregory ('13, p. 507) “. . . do not share his optimism, however, and can only say that while his results are remarkable, his race is not yet dead. Is there any clue to the particular make up of this race of *Paramecium aurelia*?” Accordingly Calkins sought the explanation of the diametrically opposite results derived from his and from Woodruff's cultures of *Paramecium* in variations in the tendency to conjugate which have been observed by Jennings ('10) and himself to exist in different races of this organism. Thus he emphasized the fact that he could readily induce conjugation in his culture whereas experiments to secure conjugation in Woodruff's culture were without effect. Calkins, therefore, stated that “the two races cannot be compared in regard to vitality, since normal conjugation was prevented in the conjugating race, whereas in the non-conjugating race there has been no artificial prevention of a normal process.” “Woodruff's *Paramecium aurelia* is evidently a *Paramecium Methuselah* belonging to a non-conjugating line the life history of which is not known in any case.”

However, conjugation was finally secured in a mass culture seeded from Woodruff's race ('14) thus demonstrating that this race is a conjugating race when the proper conditions for its consummation are realized. Therefore, there is no evidence extant that a non-conjugating race of *Paramecium* exists.

With this theory swept away by a fact the results derived from this culture stand where they stood before and demonstrate that the very limited periods in which Maupas, Calkins, and others observed degeneration have no significance for the question as to whether degeneration and death are inevitable results of reproduction without conjugation. In other words, this one positive result from Woodruff's race outweighs all the negative evidence derived from work on the Infusoria, and justifies the statement that these organisms can live indefinitely, when subjected to favorable environmental conditions, without conjugation or artificial stimulation.

Although morphological or physiological variations which could be interpreted as the result of degeneration were never observed in Woodruff's race of *Paramecium*, it was early noted ('08) "that various nuclear changes which are not at present recognized occur normally in the life history of *Paramecium*," and suggested that possibly when conjugation is prevented a reorganization of the nuclear apparatus within the individual cell occurs. Erdmann ('08) independently reached an essentially similar view from a consideration of the published data on this culture and a critical study of infusorian life histories, and further, in an experimental study of *Amoeba diploidea* ('13), suggested that a relation exists between sexual phenomena and rhythms. Accordingly the present authors have collaborated in a study of the daily cytological changes of this race of *Paramecium aurelia* (I).

As a result of our study of a large series of animals preserved daily during the past half year and of specimens preserved at various periods throughout the existence of this culture of *Paramecium*, we have discovered that the rhythms in the division rate are the physiological expression of internal phenomena which involve the formation of a complete new nuclear apparatus, by a definite sequence of normal morphological changes which simulate conjugation. This nuclear reorganization, in essence, consists of a gradual disintegration and absorption of the macronucleus in the cytoplasm. Simultaneously a multiplication of the micronuclei is in progress. Certain of the resulting micronuclei degenerate while the remaining one or two form the new



Text fig. 1 Diagrammatic survey of the reorganization process. Descending phase, 1-5; climax, 6-9; ascending phase, 10-14. Dot in circle = micronucleus; isolated dot = degenerating micronucleus; lines and dots = macronucleus; stipple = chromatin body; crosses = macronuclear anlage.

macronuclear and micronuclear apparatus. This results in the reorganization of the cell without the fusion of two animals (text fig. 1).

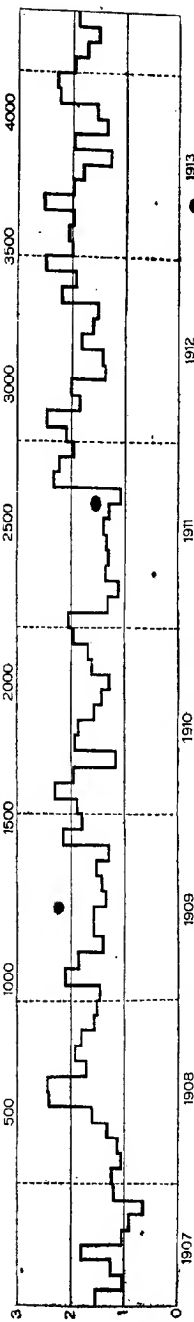
II. MATERIAL AND METHODS

A. METHODS OF CONDUCTION OF THE MAIN CULTURE I

Since the results presented in the present paper are based on a cytological study of the main pedigreed race (I) which has been carried on for the past seven years, a brief outline of the methods employed in its conduction must be given. For further details the reader is referred to earlier papers on this race (Woodruff '08, '11).

A specimen of *Paramecium aurelia* was isolated from a laboratory aquarium on May 1, 1907, and placed in about five drops of hay infusion on an ordinary glass slide having a central depression. When the animal had divided twice, producing four individuals, each of these was isolated in fresh media on a separate slide, thus forming the four main lines (Ia, Ib, Ic, Id) of this culture, *Paramecium aurelia* I. This pedigreed race has been maintained by the isolation of a specimen from each of the four lines practically every day during the more than seven years of its existence. The isolations have been made with a capillary pipet and a Zeiss binocular microscope, oculars 2, objectives 55a. This daily isolation has prevented the possibility of conjugation occurring, afforded fresh culture medium, and enabled an accurate record of the generations to be kept. Therefore this is a pedigreed culture of *Paramecium*. The rate of division of the culture has been determined by averaging the daily rate of these four lines and the accompanying graphs show this again averaged for various periods as indicated in the respective legends (text fig. 2).

The culture material supplied to the main lines of the race has consisted of thoroughly boiled infusions of materials taken practically at random from ponds, swamps, etc., in an endeavor to supply the general type of material ordinarily met with by this organism in nature. The depression slides holding the animals have been kept in a glass moist chamber on a laboratory table and therefore the organisms have been subjected to the ordinary fluctuations of temperature, light, etc., of the room. The morphological condition of the animals has been followed



Text fig. 2 Graph showing the rate of reproduction of the pedigree race (I) of *Paramaecium aurelia* from start on May 1, 1914, at the 4394th generation. The ordinates represent the average daily rate of division of the four lines of the culture again averaged for each month of its life to date. The vertical broken lines indicate the limits of the calendar years. The figures 500, 1000, etc., represent generations and are placed above the periods in which they were attained. (The fluctuations in the curve are chiefly the result of the varied culture conditions and therefore must not be confused with rhythms.)

in a general way by periodically preserving specimens left over at the daily isolations.

The culture was started and conducted for two months at the Biological Laboratory of Williams College, and since has been carried on at the Biological Laboratories of Yale University, except during a part of each summer when it was transferred to the Marine Biological Laboratory at Woods Hole, Mass.

It is under these conditions then that this culture (I) has been successfully maintained without loss of vigor for over 4500 generations—all the experiments which have been conducted on animals from this race having been made on sub-cultures started from the animals left over from the lines of this culture at the daily isolations.

B. METHOD OF CONDUCTION OF SUB-CULTURE IE

For the intensive study of the daily cytological changes of this race of *Paramecium aurelia*, on October 27, 1913, six animals left over from Line Ia of the main culture at the 4020th generation were isolated to start a new sub-culture, designated IE, of six lines. This sub-culture was subjected to practically constant environmental conditions. The culture medium was the 0.025 per cent beef extract which Woodruff and Baitzell ('11 a) found to be a most favorable medium for this race of *Paramecium*. The sub-culture was kept in a thermostat set for 26°C., and such variations (about 1°C.) from this as occurred were well within the optimum zone for the animals of this race as determined by Woodruff and Baitzell ('11 c).

An animal was isolated every day from each line of this sub-culture from its initiation to April 27, 1914—a period of six months. The isolated animal from each line was placed in fresh culture medium, while one or more of the remaining animals was preserved for study. Thus for half-a-year permanent preparations were made from day to day of sister cells, the exact ancestry of which was known in every case. *It is important to emphasize the fact that by this method only, i.e., the study of practically each cell generation, could the sequence of nuclear changes which we describe be determined.* This became evident very soon as the

work proceeded and consequently the study was concentrated particularly on one (VI) of the six lines—the other lines being merely controlled to show the occurrence of the process, at which time all the cells available from these lines were also preserved. In many cases all the animals of certain lines were killed in the reorganization process so that every animal could be studied. The places of these lines were supplied by new ones from one of the remaining five lines. Thus by killing of various lines and the branching of others to take their places a total of fifteen sub-lines of longer or shorter duration were formed. These together with the six original lines made a total of twenty-one lines whose cytology was investigated.²

The individual animals were fixed in Schaudinn's sublimate-alcohol (stronger solution), stained with Delafield's hematoxylin and mounted in cedar oil—the specimen being watched under a Zeiss binocular microscope throughout the operations, and transferred with a capillary pipet from one depression slide to another as occasion demanded. Differentiation was effected with acidulated alcohol (70 per cent alcohol + 0.002 per cent HCl) under a compound microscope. The following staining reagents were tried but the above methods gave the best results—bearing in mind that each animal had to be carried along under constant observation through each of the fluids until it was in the cedar oil under a coverglass: Heidenhain's hematoxylin and Bordeaux red, methyl green and eosin; Delafield's hematoxylin and eosin; safranin and methyl green; Mannsche Färbung and Giemsa feucht. Animals preserved in bulk from mass cultures were submitted to the same staining methods and again Delafield and eosin were found most satisfactory; Heidenhain staining the trichocysts too greatly. Sections (5μ) were made of animals in various stages of the process but they did not afford important details which could not be made out in the total mounts. The sections were stained with safranin and Heidenhain according to the suggestions of Enriques ('12).

² After the definitive experiments were formally concluded at the end of six months, certain lines were continued to June 14, 1914, in order to secure some further details.

In addition to this material preserved daily from Sub-culture IE, we had at our disposal, as already mentioned, preparations preserved at various periods throughout the life of the main culture (I). These had been fixed in a saturated solution of corrosive sublimate with 5 per cent glacial acetic acid, stained with Ranvier's picrocarmine and mounted in damar.

III. DESCRIPTION OF THE CYTOLOGICAL CHANGES IN THE REORGANIZATION PROCESS

The general outline of this remarkable cytological process which accompanies the rhythms clearly shows that a complete internal reorganization of the *Paramecium* cell occurs without cell fusion. The details to be presented were obtained as already described from Sub-culture IE, from October 27, 1913, to April 27, 1914, and certain stages were substantiated with specimens which had been preserved from the main culture (I) at various isolated periods during the previous six and one-half years of its existence. Figure 1 (pl. I) shows a typical specimen of *Paramecium aurelia*, isolated from the main culture (I) on April 11, 1908, at the 424th generation, i.e., 3596 generations before Sub-culture IE was started. Figure 2 (pl. I) represents an animal in the 4020th generation at the time Sub-culture IE was branched from the main Culture I.

The reorganization process resolves itself naturally into three periods: the descending phase, the climax, and the ascending phase.

A. DESCENDING PHASE

The macronucleus of a typical *Paramecium aurelia*, which is not undergoing the reorganization process, consists of fine chromatic granules enclosed within a relatively thick membrane. The finer structure has been fully described by Maupas ('89, p. 217) and Hertwig ('89, p. 9). The two micronuclei, in a similar period with respect to the process and in the resting stage between two cell divisions, are more or less compact and homogeneous, and often lying in a pair near the macronucleus which sometimes obscures them from view. The cytoplasm of the cell

contains many small vacuoles some of which may be filled with bacteria (figs. 1 and 3, pl. 1).

As the low point of the division rate approaches, the granules of the macronucleus become more and more coarse, its staining capacity increases, its form becomes more kidney-shaped and its shorter axis elongates. Still more obvious changes of the macronucleus indicate the actual beginning of the regulation process.

1. *The macronucleus*

At this stage projections appear at the end of the macronucleus (figs. 4 and 5, pl. 1) which are either merely thin membranes devoid of chromatin or filled with small granules. Hertwig ('89, p. 7) describes them as follows: "gleichzeitig verliert die Oberfläche ihr glattes Aussehen; Einkerbungen erstrecken sich mehr oder minder tief in das Innere hinein und zerlegen den Kern nicht selten in drei ungleich grosse Lappen oder es werden an den Enden fingerförmige Fortsätze deutlich oder leisten- und riffartige Vorsprünge." According to this author they are not evident during the latest stages of vegetative cell division, or in the first changes incident to conjugation. Though Hertwig could not observe these projections in the living animal he did not believe them to be artifacts because he could demonstrate them with every fixation fluid. We have never observed them in vegetative cell divisions but frequently have noted their presence several generations before the definite onset of the process. We have no data in regard to their relation to conjugation.

* Figure 5 (pl. 1) gives a good idea of these projections. The animal figured is from Line VI, 4094th generation, and is nine generations before the beginning of the reorganization process which started in the 4103d generation. These long finger-like projections are preceded by the appearance of smaller ones (fig. 4, pl. 1) which seem to indicate that plasmatic currents are present in the macronucleus though the membrane at this stage is still intact.

The next stage which is peculiar to the process is the separation of chromatin bodies from the macronucleus. In the early stages of the reorganization process cells are seen with only two or more chromatin bodies (figs. 6 and 7, pl. 1) which are more or less spherical and consist of large and small granules. Figure 8 (pl. 1) shows a macronucleus which is surrounded by several of these bodies which have been ejected from it. This method of nuclear disintegration is not unique in Infusoria because similar morphological phenomena are described by Neresheimer and Buschkiel. Neresheimer ('08) mentions that chromatin bodies are ejected from the macronucleus of *Ichthyophthirius* before the beginning of the sexual process. This species during vegetative life has its macronucleus and micronucleus in one body, but this difference is unimportant because the chromatin bodies of *Ichthyophthirius* contain the combined material of the macronucleus and micronucleus, and at the time of extrusion the vegetative or sexual character of the chromatin bodies is determined. In *Paramecium aurelia* merely vegetative nuclear material is extruded by the macronucleus.

A connection between the macronucleus and the chromatin bodies of *Paramecium* as described by Buschkiel in *Ichthyophthirius* ('08, p. 81) could not be discovered and seems not to exist because these bodies are formed in the macronucleus and are ejected through openings in the membrane. The formation of the chromatin bodies begins in the macronucleus and is evident by the condensation of granular material at various points within the membrane (fig. 8, pl. 1), other parts of the macronucleus becoming devoid of chromatin as segregation proceeds. The chromatin bodies are not yet surrounded by clear areas. The resulting membranous condition of the old macronucleus is shown in figures 6, 10 and 11 (pl. 1) and figure 14 (pl. 2). Two characteristic stages in the elimination of chromatin bodies by the macronucleus are given in figures 8 and 9 (pl. 1).

The chromatin bodies are removed from their place of origin by movements of the cytoplasm, thus emphasizing the well-known cytoplasmic currents in the cell. The specimen shown

in figure 10 (pl. 1) has seven of these bodies arranged in a semi-circle at the posterior end of the cell. The anterior end contains some irregularly shaped chromatin bodies which seemed to be undergoing involution, while three others more recently ejected are still near the macronucleus. The disintegration of the macronucleus progresses until it is entirely devoid of chromatin (figs. 35, 36, pl. 4) while the wrinkled and ruptured membrane with but slight staining capacity remains in the cell (fig. 14, pl. 2.) In the later period of the reorganization process the membrane is resorbed and the old macronucleus has finished its function in the paramaecium cell.

These changes of the macronucleus have their analogy in normal conjugation. There the old macronucleus is destroyed but this is effected by the formation of the so-called "wurstförmige Schlingen," which form a tangled mass of chromatin ribbons. Their origin is figured by Maupas (fig. 10, pl. 12; figs. 17-20, pl. 13); and by Hertwig (figs. 6-9, pl. 1; figs. 1-9, pl. 2) and in the present paper (fig. 44, pl. 4). However, in conjugation it is not until after the animals have separated that the chromatin ribbons are totally fragmented and more or less spherical bodies are free in the cell. Later an involution of these occurs, the details of which are not described by either Hertwig or Maupas. Likewise, the persistence of the membrane of the macronucleus is not figured by these authors. This ribbon-like formation of the macronucleus we have found to be characteristic of conjugating animals from this race of *Paramaecium aurelia* (fig. 44, pl. 4). Figure 32 (pl. 3) shows a pair of conjugants with their macronuclei in this form.

In this period then the differences between the macronuclear changes during conjugation and during the process are only morphological; on the one hand, the macronucleus forms 'wurstförmige Schlingen;' on the other the macronucleus eliminates its chromatin by extruding it in the form of spherical bodies. The physiological effect is the same. The result is the destruction of the old macronucleus.

2. The micronucleus

While these changes are taking place in the macronucleus the micronuclei do not remain unaltered. They move from their accustomed position, which is more or less close to the macronucleus, and migrate in the cytoplasm (fig. 3, pl. 1). The same appearance was noted in *non-conjugating* *Paramecium caudatum* by Calkins and Cull ('07, p. 383), who state that in stages of a pedigreed race, which Calkins calls depression periods, the micronucleus migrates in the cytoplasm, and they interpret this as an abnormal condition. In *conjugating* animals the migration of the micronuclei at the beginning of the sexual process is described in *Paramecium aurelia* by Maupas ('89, p. 212) and by Hertwig ('89, p. 21); in *Paramecium caudatum* by Calkins and Cull ('07, p. 383); and in *Paramecium bursaria* by Hamburger ('04, p. 200). This migration seems to be a general phenomenon at the onset of the changes incident to conjugation and occurs also, as described above, in isolated paramaecia before the beginning of the reorganization process.

The micronuclei in their new position proceed to divide with the result that finally eight are present in the cell. Figure 14 (pl. 2) shows a specimen in which there are two solitary micronuclei and two groups of three micronuclei. All the micronuclei show a perfectly normal structure but some variation in size is evident. In typical conjugating animals the eight so-called reduction micronuclei are described by Hertwig and Maupas as all being of the same size, but Maupas did not lay great stress on cytological details so that only Hertwig's statement is of weight. It is probable that the differences of volume shown by micronuclei in the process are due to variations in the amount of nuclear sap preliminary to the cessation of their functional activity.

It is impossible to determine whether these micronuclei of the animals in the process are actually reduction micronuclei because, here, as in the conjugation of *Paramecium aurelia*, it is not possible to count the number of chromosomes.

The formation of the eight micronuclei does not begin until the disintegration of the macronucleus is about finished. Figures 36 and 37 (pl. 4) illustrate the coincidence of the end of the macronuclear disintegration and the beginning of the multiplication of the micronuclei. The animals in figures 35 and 36 show the typical form of the macronuclear destruction which takes place in the reorganization process without the formation of 'wurstförmige Schlingen'. A slight resemblance to the characteristic macronuclear condition during conjugation is given in the isolated paramaecium of the 4087th generation, thus indicating that the macronuclear destruction in conjugation and in the reorganization process may sometimes show similar features. The animals shown in figures 36 and 37 possess either three or four 'reduction' micronuclei. The identification of these as 'reduction' micronuclei is based upon their position in a homogeneous protoplasmic layer, and the absence of a micronuclear membrane, together with the general morphological structure of the cell and its fate.

The diagnostic characters of the 'reduction' micronuclei are still more prominent in later stages. Figure 11 (pl. 1) shows a macronucleus partly devoid of chromatin, eight chromatin bodies and three micronuclei. Two of the micronuclei are apparently starting to divide. The anterior micronucleus seems to be in the process of forming another by an unequal distribution of the chromatin inside the membrane. This is the only micronuclear change of this type which we have observed and may well be due to some irregularity. The formation of mitotic spindles with long distinct threads and crescents have not been seen, and apparently exactly the same type of mitosis as described by Hertwig does not occur in the reorganization process. However, figure 31 (pl. 3) illustrates an animal, from a sub-culture of this race in which conjugation was allowed to occur, having division spindles which approximate to a certain degree some of the spindles figured by Hertwig. It is well known that the morphology of the mitotic apparatus varies at different phases of cell life, as for example was found to be the case in *Amoeba diploidea* by Erdmann ('11, p. 336).

Calkins and Cull ('07, p. 383) note that, during periods which they call depressions, the typical structure of the micronucleus of *Paramecium caudatum* is lost and it becomes abnormally large with the chromatin in a loosely granular condition. It is a remarkable coincidence that this occurs also in *Paramecium aurelia* in the descending phase of the process.

Figure 12 (pl. 1) shows the macronucleus, which now has become smaller, together with fourteen chromatin bodies free in the cytoplasm, and two just leaving the macronucleus. The cell possesses three micronuclei: two, lying near each other in a cytoplasmic layer free from granules, are in early division stages, while the third has nearly finished division. Thus the cell soon would have contained six micronuclei. Though four micronuclei are formed in normal vegetative division the beginning of the 'reduction' division is distinguishable from that of vegetative division because the four vegetative micronuclei are usually either lying in closely associated or widely separated pairs in the cell (fig. 7, pl. 1) while the 'reduction' micronuclei remain clustered together in a layer of homogeneous cytoplasm. Later stages showing seven and eight micronuclei are given in figure 13, pl. 1, and figure 14, pl. 2, already described. Thus at the end of the descending period of the reorganization process the *paramecium* cell possesses eight micronuclei, a shrunken membrane of the old macronucleus, and numerous chromatin bodies which have passed out from the macronucleus (fig. 14, pl. 2).

The important multiplication of micronuclei is not without precedent in non-conjugating animals. Maupas mentions a non-dividing animal with three micronuclei. Popoff ('09, p. 30) describes a specimen of *Paramecium caudatum* with two micronuclei which are just preparing to divide and form four without cell division ensuing. Also Kasanzeff and Calkins note the multiplication of the micronuclei in *Paramecium caudatum* without cell division. Popoff states further that in *Stylonychia mytilus*, after treatment with a medium with CO₂ in solution, the normal four micronuclei (p. 15) multiply until eight may be present. It is unnecessary at this point to discuss the theoretical

bearings of these cases (cf. p. 483) but merely to emphasize the fact that the multiplication of the micronuclei in an isolated cell is *not* an infrequent event, and that the fate of the cell alone gives the significance of the phenomena.

B. CLIMAX

The period of the reorganization process, designated the climax, is the most important of the three phases. Morphologically defined it extends from the total disintegration of the old macronucleus to the formation of the new macronuclear anlagen. Physiologically speaking it represents a stage in which the vegetative functions of the cell are relatively in abeyance and only the potentialities of the micronuclei are in evidence. At the end of the descending phase the paramaecium cell possesses eight micronuclei, twenty to thirty spherical chromatin bodies and the membranous remains of the old macronucleus (fig. 14, pl. 2). Figure 13 (pl. 1) gives an animal just as the degeneration of the micronuclei sets in. It shows six micronuclei, one of which is dividing, scattered in the cell, and numerous chromatin bodies but no trace either of the old macronucleus or of the macronuclear anlagen. Four micronuclei are closer together than the other three, indicating that the two groups arise from each of the two original vegetative micronuclei. The scattered position of the micronuclei indicates the beginning of their degeneration (see fig. 44, pl. 4) which shows the same feature in a conjugating animal.

The next change consists in the practically complete disappearance of the old macronuclear membrane and in the degeneration of the so-called reduction micronuclei to the number two (or one). It was not possible to trace the method of degeneration of the micronuclei but it is positive that in the generation which follows the above described stage only one or two are present. Maupas and Hertwig figured the degenerating micronuclei in *conjugating* animals as homogeneous minute dots the structure of which is not discernible (Hertwig '89, fig. 7, pl. 1; Maupas '89, fig. 13, pl. 12). Hertwig at first could not determine

whether one or two of the reduction micronuclei remained and formed immediately without a further division the stationary and migratory nucleus, but in his final publication ('89, p. 31) he states definitely that seven reduction micronuclei degenerate but his *figures* do not absolutely prove this point (fig. 6, pl. 1; fig. 24, pl. 4). A micronucleus remains which is the source of the new micronuclear and macronuclear apparatus. Maupas ('89, p. 219) stated earlier than Hertwig that "Sept d'entre eux vont, en effet, passer à l'état de corpuscles de rebut et disparaître en se résorbant. Un seul persistera et continuera l'évolution fécondatrice," but as already mentioned, he does not give cytological details so that a perfect description of the degeneration of the micronuclei in *Paramecium aurelia* does not exist. It is easier to trace the degenerating micronuclei in *conjugating* animals because there the ribbon-like formation of the macronucleus is still intact (fig. 44, pl. 4). Many chromatin bodies are not free in the cell and consequently cannot be mistaken for degenerating micronuclei. Thus each tiny homogeneous granule in the cytoplasm can be identified as a micronuclear remnant, whereas in the reorganization process unconnected chromatin bodies are scattered freely in the cell and minute homogeneous granules intermingled with them may or may not be identified as degenerating micronuclei. However, certain bodies which have been observed we are inclined to interpret as micronuclear remnants. These will be indicated in the description of the plates.

The reorganization process proceeds in two different ways, as shown by the animals which were preserved at the climax, depending upon whether or not a *cell division* takes place at this period. The usual method is for a cell division to occur. In the cell which possesses no macronucleus the chromatin bodies change their shape, becoming more and more elliptical while each granule begins to migrate to the surface so that the bodies appear hollow. The one remaining micronucleus is large. Figure 15 (pl. 2) shows such cells which have just completed the division at the climax. Each has one micronucleus while the anterior cell has eight and the posterior twelve chromatin bodies.

The posterior animal still contains a piece of the old macronuclear membrane. It is possible that the two micronuclei in this dividing animal (fig. 15, pl. 2) are the result of a division of a single 'reduction' micronucleus which alone remained in the parent cell. It is also possible that *both* of these micronuclei are the 'reduction' micronuclei which did not degenerate and which are now, after a long period of passivity, distributed to each of the daughter cells. In this case the formation of a new spindle could not have occurred and the absence of the micronuclear division would be proof that the process is effected in this way. In eight cases of the process the cytological study has shown that a cell division actually does occur. Such cases fall under the heading Ia and Ib in text figure 3.

The discussion of the micronuclear events preceding the cell division in the climax will be postponed until all the cytological data from the study of the climax have been presented.

The morphology of the cell *after* the cell division in the climax was definitely determined from several specimens (see fig. 15, pl. 2, and figs. 41 and 40, pl. 4). Clearly the reorganization of the nuclear apparatus is effected by a single micronucleus in all cases in which a cell division occurs in the climax. Figures 41 and 42 (pl. 4) give two cells in the 4436th and 4437th generations respectively. Cell 4436 has one micronucleus and numerous chromatin bodies and is an animal *after* the cell division in the climax. Cell 4437, which is a product of the first division of the sister cell of cell 4436 after about forty-eight hours, already has one macronuclear anlage. This proves that, *after* the discussed cell division in the climax, the one micronucleus forms, by two subsequent divisions, four micronuclei, two of which become macronuclear anlagen which are distributed by the succeeding cell division to each of the two daughter cells.

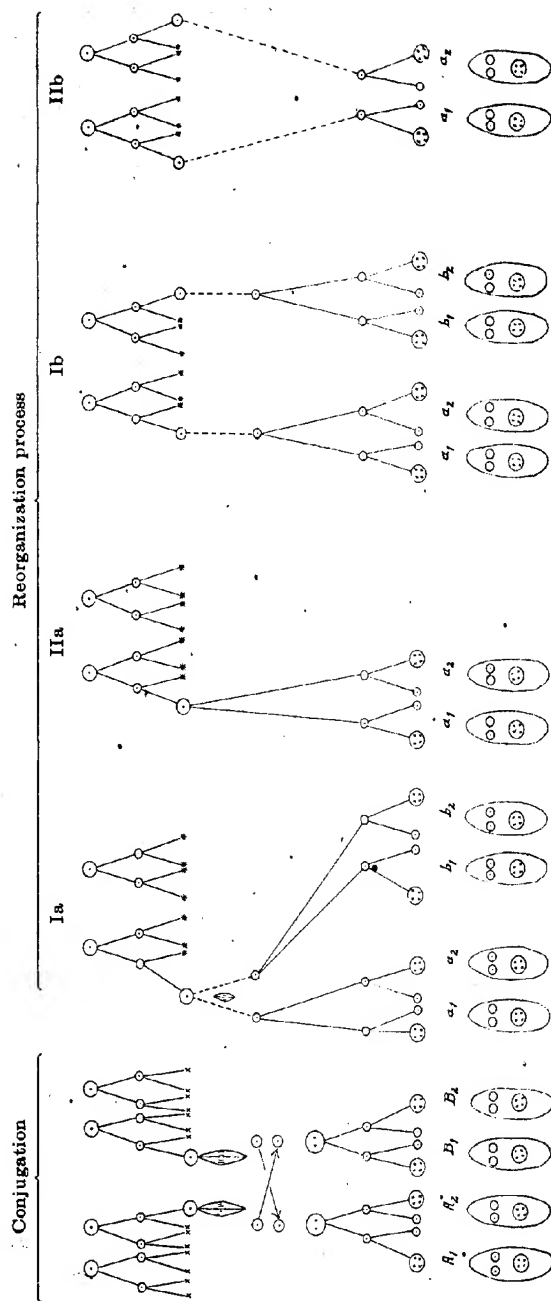
In figure 16a (pl. 2) is given a more advanced stage of one of the cells immediately after division. This anterior cell (a) has just emerged from the climax, as is evident from the fact that the formation of the macronuclear anlagen is completed. The sister cell (b) has not undergone the same changes and one micronucleus and several chromatin bodies, of which only five

at the anterior end are figured, can be detected. Figure 39 (pl. 4) shows essentially the same stages, the only difference being that the anterior cell has effected one more micronuclear division before the first reconstruction cell division than 16a.

While the morphological changes which occur after the cell division at the climax are clear, those which take place before this division (i.e., from the formation of eight 'reduction' micronuclei to the distribution of two to sister cells) must be discussed further. In the instances where two micronuclei remain, it is possible that a micronuclear division has not taken place, but that merely a shifting of one of the micronuclei into each of the two newly formed sister cells has occurred. This method of distribution, which gives an equally satisfactory explanation of the observed data, we call Case Ib (text fig. 3). The results of cases Ia and Ib are the same—two cells with one micronucleus and many chromatin bodies at the climax.

Only twice during these experiments has a paramaecium been observed which had a macronucleus half devoid of chromatin, two well formed micronuclei, two macronuclear anlagen and several chromatin bodies. In these cases the latter were so small and homogeneous that they readily could be mistaken for 'reduction' micronuclei. Here it is evident that no division during the climax took place and that the formation of the anlagen has occurred precociously. There are thus two possibilities as to the *origin* of this condition: (a) *One* so-called reduction micronucleus may remain and divide. The two micronuclei thus arising form the anlagen (text fig. 3, IIa). (b) *Two* so-called reduction micronuclei may remain and divide, thus forming immediately the macronuclear anlagen (text fig. 3, IIb). The result in either case is the same—one cell with two micronuclei and two macronuclear anlagen.

Data will later be presented (cf. p. 495) which afford strong *physiological* evidence that a third micronuclear division producing gametic micronuclei (stationary and migratory) must be absent. It is important to note that if no micronuclear division takes place between the last so-called reduction division and the divisions which form the new micronuclei and give rise to the



macronuclear anlagen (Ib, IIa, IIb), a primary distinction between the morphological phenomena in conjugation and in the process here described is established, i.e., the important micronuclear division which forms the migratory micronucleus and the stationary micronucleus is absent. But we wish to emphasize the fact that if a third micronuclear division occurs, it can equally well be a precocious division in the ascending or reconstruction phase of the reorganization process.

The diagram presented herewith (text fig. 3) summarizes the different cases which are possible from the observed facts. *All the described cases are distinguished from conjugation and autogamy by the absence of the formation of a syncaryon.* In the process only in case Ia already described is it possible that a third micronuclear division at the climax takes place. The difference between conjugation and the process in case Ia consists in the occurrence of one more *cell* division in the latter than in conjugation. The possible variations in cases Ib, IIa and IIb agree in the absence of the third micronuclear division under discussion. Cases IIa and IIb have no cell division before the formation of the macronuclear anlagen, one or two so-called reduction micronuclei remaining in the same old cell. Case IIb is peculiar in the shifting of one micronucleus into each new cell.

But from a study of all the observed sequences of generations at this period there is some evidence that case Ib presents the usual method of nuclear changes at this period. Line VIId had four and Line VIb had three animals in the reorganization process, all of which were at the first stage of the ascending phase—formation of the macronuclear anlagen. This one fact is crucial evidence that a *cell division* has occurred before the formation of the macronuclear anlagen, but no direct *observation* can be presented which determines whether the discussed micronuclear division has or has not taken place, because one spindle in a cell filled with chromatin bodies without a macronucleus or with a macronuclear membrane devoid of chromatin can represent either: (a) the discussed third micronuclear division or (b) the division which forms in cases Ia, Ib, and IIa the two new micronuclei which in the next division form the macronuclear anlagen.

Figure 40 (pl. 4) shows an actual cell to illustrate these statements. The animal has many chromatin bodies and two micronuclei which have just divided. Only because the *fate* of the cell (VIh, 4355th generation) is known, is it certain that these two micronuclei are the two micronuclei which, by another micronuclear division, form four micronuclei, two of which become macronuclear anlagen. If the cytology of the cells related to this cell were not known, its two micronuclei could be interpreted as the products of the discussed third division, which, if it occurs, should take place *before* the cell division in the climax. *We have in our pedigreed lines no indication of its occurrence.*

Only on the assumption that this important division in the climax has different morphological structures from the other divisions of the reorganization process would it be possible to recognize it. However since we have only seen small elongated spindles during the *ascending* phase there is no reliable morphological criterion to determine the presence of the third micronuclear division. One might expect that the condition of the chromatin bodies would give some indication of the extent to which the process has advanced and therefore of the number of micronuclear divisions which has occurred. But this is not a positive criterion because the disintegration of the chromatin bodies does not progress with equal rapidity in all cells.

We are certain, from the *combined* evidence from our cytological study and from physiological data which will be presented later, that the micronuclear division which would be comparable to that which forms the stationary and migratory micronuclei in conjugating animals is absent in the reorganization process. In *conjugation* the reorganization of the nuclear apparatus is consummated by the syncaryon. In the *reorganization process* the so-called reduction micronuclei or their descendants give rise to the new nuclear apparatus. This significant feature is obviously of great importance from the standpoint of the theoretical interpretation of the reorganization process which we describe.

A brief survey of all the stages from the climax which we have figured in the plates substantiate, we believe, the cytological

data which we present and our interpretations of them. The third micronuclear division is absent; two 'reduction' micronuclei remain which are shifted into two cells. Figures 15, 16 and 39 show the cell division in the climax; figure 41 gives a single cell which has completed that division; figure 40 shows the next micronuclear division preceding the formation of the macronuclear anlagen; figure 16, anterior cell, figures 17 through 24, and figure 39, anterior cell, give the formation of the two macronuclear anlagen and the micronuclear changes in each stage. The distribution of the macronuclear anlagen is shown in figures 25, 42, and 43.

C. ASCENDING PHASE

The ascending phase of the reorganization process, which is the longest of the three, extends from the formation of the macronuclear anlagen to the restoration of the typical paramaecium cell with one macronucleus and two micronuclei. It is identical with the periods F and G which Maupas describes as the first divisions after typical conjugation in *Paramaecium aurelia* ('89, p. 221).

Two micronuclear divisions occur in a very short time, half the products of the second forming the two macronuclear anlagen. The two untransformed micronuclei divide again and the first cell division ensues. The following divisions of the cell are exactly similar to typical vegetative divisions and can only be distinguished by the fact that the involution of the chromatin bodies is in progress.

The details of this period are remarkable in various ways. Figures 17, 18, and 19 (pl. 2) give details of the formation of the macronuclear anlagen. The chromatin bodies are omitted from the drawings. These three preparations are counter-stained with eosin, which, according to Calkins ('07, p. 383), stains the non-chromatic parts of the micronuclei of *Paramaecium caudatum*. The posterior micronucleus (fig. 17) has just divided and of the products of this division one remains a typical micronucleus while the other shows the beginning of the development of a micronucleus into the fundament of the macronucleus.

Chromatin has just separated from the achromatic threads but still retains its micronuclear structure. The next step in forming the anlagen is the distribution of the threads and the arranging of the coarser chromatic granules underneath the membrane. This step has been effected in the anterior anlage shown in the same figure. Figure 18 gives a clear idea of the completed anlagen and the two micronuclei from which they arose. Here the granules have become smaller and the plasmatic character of the structure is more evident because its staining capacity for eosin has increased. The micronuclei show the chromatin surrounded by a plasmatic layer highly stained with eosin. In figure 19, the next division, which is the first in the new rhythmic period of the life history, is completed in one pair of micronuclei and is in progress in the third micronucleus which is lying just above the one chromatin body shown in the figure, though many were present in the cell. All the chromatin bodies are represented in an animal from Line VI (fig. 21, pl. 2). Nearly all are more or less spherical and the dissolution of the chromatin is evident. Two macronuclear anlagen, lying close together, and stained a reddish-blue, show marked paucity of chromatin. The two functioning micronuclei are lying at the opposite ends of the cell, while a spindle, in which chromatin has passed to the two poles, is also visible. This spindle represents the same formation as that figured by Maupas in a conjugating animal ('89, pl. 13, fig. 27). Above one of the chromatin bodies is seen a small chromatin granule which may be interpreted as a degenerating micronucleus.

The disintegration of the chromatin bodies, which are now often surrounded by a clear area, is also progressing in the animal represented in figure 20 (pl. 2). The macronuclear anlagen have lost their round contour and appear more or less irregular while the two micronuclei are ready for division. The micronuclear divisions are far more advanced in the specimen shown in figure 22 (pl. 2) and the two spindles present indicate an ensuing cell division. One micronucleus is apparently degenerating. Large vacuoles are usually present in the cytoplasm during this period.

The last period of the ascending phase is characterized by the disappearance of all chromatin bodies. Figure 25 (pl. 3), Line VIb, represents an animal, after the first cell division following the origin of the macronuclear anlagen, showing two micronuclei at the left, twenty chromatin bodies and several large vacuoles. A single new macronucleus is visible. The other macronuclear anlage, of the two in the parental cell, has been distributed to the sister cell which was kept to continue Line VIb. This method of distribution of the two macronuclear anlagen to each of the new animals is the same as in typical conjugation of *Paramecium aurelia*. Maupas ('89, p. 222, pl. 13, figs. 23-27) gives a full account of this distribution. Hertwig ('89, p. 38) shows that one of the products of each of the two dividing micronuclei becomes a macronuclear anlage and the other a micronucleus, but this is not evident from his figure (pl. 3, figs. 9 and 10). The destruction of the old macronucleus occurs in most Infusoria during and after conjugation by the formation of ribbon-like structures which are finally resolved into chromatin bodies and disappear. This is the method which obtains in *Paramecium caudatum* and *Paramecium aurelia* (Maupas, Hertwig) and in *Paramecium putrinum* (Doflein). But the accounts of this stage given by Maupas, Hertwig, and Calkins do not afford details of the ultimate fate of the chromatin bodies, since they merely mention that, by the formation of the daughter cells, these bodies decrease in number and their remnants become pale and disappear in the cytoplasm. Collin ('12, p. 223) however, gives a thorough account of the old macronucleus in *Acineta papillifera* and figures (text-fig. 63) the gradual resorption of the chromatin remnants in the cytoplasm. A different fate of the macronucleus was observed by Ubisch ('13, p. 72) in a study of *Lagenophrys*, who states that the chromatin bodies, which appear very similar to micronuclei, are actually ejected from the macrogamete after the formation of the syncaryon. The extruded chromatin bodies were finally observed between the animal and its test.

The total disappearance of the chromatin bodies in the reorganization process of *Paramecium aurelia* is thoroughly described on page 455. It is distinguished from the similar stage in con-

jugation by the fact that chromatin ribbons are not formed. There is no evidence that the chromatin bodies are ejected from the cell or that the remnants fuse with the new macronucleus. After the reorganization process the totally dissolved material of the old macronucleus probably remains, at least temporarily, in a changed chemical form in the cell.

From our studies of conjugation in animals derived from the main culture of the pedigreed race of *Paramaecium aurelia* we can show clearly that the first division after conjugation distributes to each cell two micronuclei and one macronuclear anlage (fig. 33, pl. 3). There is no degeneration of nuclear material as Maupas ('89, p. 204) and Klitzke ('14, p. 8) have described in *Paramaecium caudatum*. Maupas as well as Klitzke state that eight micronuclei are formed from the syncaryon by two mitoses quickly following each other, four of the resulting micronuclei forming the macronuclear anlagen, three degenerating and one remaining as the definitive micronucleus of the reorganized cell. By two cell divisions, accompanied by divisions of the micronuclei, the typical vegetative stage in *Paramaecium caudatum* is restored. However, Calkins and Cull ('07, p. 387) do not mention a degeneration of nuclear material in this stage but state that four of the eight micronuclei are transformed into macronuclear anlagen and, by two cell divisions without division of the micronuclei, four typical vegetative animals result. Doflein in his account of *Paramaecium putrinum* gives the same interpretation as Maupas and Klitzke, i.e., three micronuclei degenerate and the four potential macronuclear anlagen are distributed by two cell divisions to four animals. Hamburger ('04, p. 223) states that in *Paramaecium bursaria* two micronuclei and two macronuclei are formed which are distributed in one of the two following ways to the sister cells which arise: either one micronucleus and one macronuclear anlage pass to each new cell; or one macronuclear anlage and one micronucleus (the product of an additional micronuclear division) pass to one sister cell, while one macronuclear anlage and the other micronucleus (resulting from the extra micronuclear division) passes to the other sister cell. The normal vegetative state of *Paramaecium bursaria*,

completely free from disintegration products of the old macronucleus, is often not completed before the third division. All descriptions of the behavior of the paramaecium cell of the different species after the formation of the syncaryon agree in the origin of four or eight new micronuclei, but these micronuclei resulting from the syncaryon are either all used in the further development of the cell (Calkins and Cull, *Paramaecium caudatum*; Maupas, Hertwig, Woodruff and Erdmann, *Paramaecium aurelia*; Hamburger, *Paramaecium bursaria*), or some degenerate (Maupas, Klitzke, *Paramaecium caudatum*; Doflein, *Paramaecium putrinum*). Therefore the weight of evidence at hand indicates that the latter alternative is due to a wrong interpretation. Similarly in the process under discussion in *Paramaecium aurelia* there is no evidence of a wasting of chromatin material in this period and its morphological features are identical with all statements of the nuclear changes in typical conjugation in this species.

The observations of Hamburger on *Paramaecium bursaria* prove that the first micronuclear division after the development of the anlagen may occur either in the old cell or later in the two daughter cells which arise from it. Before the first cell division in the reorganization process two spindles are formed (fig. 22, pl. 2) which indicate that the first cell division after the formation of the macronuclear anlagen restores the normal nuclear apparatus of the cell. During the next two generations the remnants of the old macronucleus are completely eliminated. This is effected by complete dissemination in the cell of the chromatin bodies (fig. 27, pl. 3) in the form of cloud-like masses of faintly staining chromatin granules which soon undergo total involution in the cytoplasm. This disintegration process does not occur simultaneously in all the chromatin bodies as is shown in the figure under discussion in which four bodies are still intact.

The animals which have undergone their first vegetative division are filled with vacuoles as shown in an animal from Line IIIb, 4313th generation (fig. 27, pl. 3). The macronucleus has assumed the peculiar spherical form which it frequently shows immediately after cell division. The climax was in the 4312th

generation and the involution of the chromatin bodies was completed in the 4316th generation. Of the eight cells which were formed by the three following divisions (4313 to 4316), four in the 4316th generation were without chromatin bodies. The preceding figure (fig. 26, pl. 3) represents an animal from Line VI, 4185th generation. Two divisions have occurred since the climax at the 4183d generation. Here the chromatin bodies which still remain have not lost their original shape, but if one considers the four division products which result from the divisions 4183 to 4185 then it is evident that a second method of decreasing the number of chromatin bodies is by their distribution to the daughter cells. As an example of this distribution the following case may be cited (cf. text fig. 8 for sister line):

Line VI	4183d generation	has 19 chromatin bodies
Line VI	4185th generation, animal a	has 6 chromatin bodies
Line VI	4185th generation, animal b	has 5 chromatin bodies
Line VI	4185th generation, animal c	was not observed
Line VI	4185th generation, animal d	was kept alive to continue the line

Two further divisions took place in twenty-four hours:

Line VI	4187th generation, animal a	has still 3 chromatin bodies (fig. 29, pl. 3)
Line VI	4192d generation	has 1 chromatin body

Therefore the ascending phase of the process can be followed in nine generations; that is, from the 4183d generation, which is at the end of the climax, to the 4192d generation which is a typical vegetative paramaecium cell except that one chromatin body still persists. Since the beginning of the process was observed at the 4182d generation, it is evident that the nuclear changes in this case extended over ten generations. The typical reduction of the division rate at the climax of the process to one division in approximately thirty-six hours occurred in this case at the 4182d to 4183d generation, and the increase in the rate to three divisions in twenty-four hours which characterizes the ascending phase of the process occurred at the 4189th to 4192d generation.

When the second or third division after the formation of the macronuclear anlagen has occurred the new macronucleus has

assumed its typical macronuclear characteristics. The elongated form is the prevailing one in the interval between two cell divisions, the broad axis is relatively short, while the few granules within the membrane are small. The appearance of an old and new macronucleus is markedly different and the two stages cannot be confused (compare figs. 3, 4 and 5, pl. 1, with figs. 28, 29 and 30, pl. 3). Figure 29 and figure 30 (pl. 3) show the new macronuclei. The animal portrayed in the former is from the above-mentioned Line VI in the 4187th generation. One large and two small chromatin bodies are visible. The latter shows a cell which was taken from the main culture in the 1201st generation. The staining capacity of the macronucleus is very marked; the micronuclei present no unusual features; and two chromatin bodies still persist. A dividing animal from the main culture at the 1432d to 1433d generations (fig. 28, pl. 3) further illustrates the distribution of the chromatin bodies. This animal has undergone four divisions during the previous twenty-four hours and is now just completing the fifth cell division, while the micronuclei have divided precociously for a sixth cell division. This affords another example of the acceleration of the division rate which is characteristic of the ascending phase of the process.

The ensuing divisions in the rhythmical period efface the last trace of the chromatin bodies and, therefore, of the reorganization process. In conjugation, as has been shown by Maupas and Hertwig, the chromatin bodies undergo the same fate at the same time. The reorganization of the cell is completed.

In the description of the process nearly all the examples presented have been animals from Sub-culture IE (4020th to 4359th generation) bred for this particular purpose under constant environmental conditions. As already described in the section on technic, animals had been preserved at irregular intervals throughout the first six years of the life of the main Culture I and on the basis of these it was early stated ('08) that profound nuclear changes occur which cannot be interpreted as abnormal and which demand further study. The character and significance of these changes obviously could only be determined by such an intensive daily study of all the available animals as the pres-

ent paper involves. It is important, however, at this point to emphasize that the process herein described is not a phenomenon peculiar to the organisms of this culture after the 4020th generation, but that it was in progress throughout the life of the race. For this purpose various specimens from earlier generations are presented in the plates. For example, figure 23 (pl. 2) represents an animal from the main culture on May 1, 1910, at the 1755th generation with its nuclear apparatus in a characteristic stage of the process, i.e., the formation of the macro-nuclear anlagen. This should be compared with figure 20 (pl. 2) which shows an animal which is a descendant of the former after a lapse of 2329 generations, i.e., it is in the 4084th generation. The similarity of nuclear conditions also is evident in figures 21 and 22. Cases such as this could be multiplied but for brevity it will suffice to note that among the preserved material there are specimens in the 426, 910, 1201, 1452, 1498, etc., generations which exhibit reorganization stages in all respects the same as those the animals have shown during the past six months of study. Consequently, when convenient, these have been figured to illustrate the process.

IV. CYTOLOGICAL DETAILS OF SERIES OF PEDIGREED CELLS IN THE REORGANIZATION PROCESS FROM SUBCULTURE 1E

In the preceding description of the cytological changes in the process the data from all lines were presented together in order to afford a composite picture of the nuclear phenomena as they appeared from the entire culture. It is the purpose of the present section to resolve this picture into its component parts in order to emphasize the exact sequence of events as actually observed in individual pedigreed series of animals from *single* lines and thus to demonstrate that our description of the process is not derived merely by combining isolated stages found at different times in the different lines, but by combining the data from a large number of *series* of animals whose genetic relation with each other was exactly known—each series showing a considerable part of the process. In combining these series there

was little chance for error for their cytological phenomena frequently overlapped.

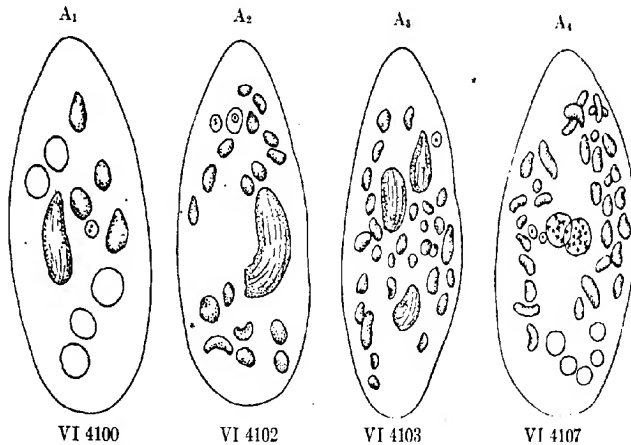
As has already been indicated, it is obviously impossible to study every animal every day and so have a complete picture of the animals of a single line at any one time, since one cell must be maintained if the line is to be kept alive. In certain cases it was deemed best to keep more than one animal alive in order to further safeguard the life of the line because death sometimes occurs during the process. However, it has been possible actually to determine the entire sequence of events by preserving all of the animals of certain lines at selected periods in the process. The fact that the process occurred nearly synchronously in the various lines and at comparatively regular intervals rendered this procedure particularly valuable.

A. SERIES OF CELLS FROM LINE VI AND ITS DERIVATIVES

Line VI at the 4057th generation afforded the first proof of the reconstruction of the cell, two characteristic macronuclear anlagen and twenty-four chromatin bodies being present. The animal is in the beginning of the ascending phase of the process. In the 4060th generation the process was completed as the animal does not show any macronuclear remnants. The large number of typical animals which followed in this line for nearly fifty divisions was finally interrupted by the appearance of a cell at the 4094th generation which has two chromatin bodies. Its sister cell (4094₂) has no signs of the beginning of the process and the following generations up to the 4102d generation showed no further traces of reorganization. This indicates that the cells when the critical period is approaching may start the process but not carry it to completion. The total reconstruction of the nuclear apparatus took place between the 4100th and 4107th generation. Text figure 4 gives two stages of the process; the disintegration of the old macronucleus in A₁ and A₂ and A₃ and the formation of the new macronuclear anlagen in A₄. In the 4100th and the 4102d generation the macronucleus is merely eliminating chromatin bodies while in the 4103d generation the

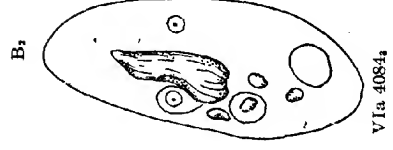
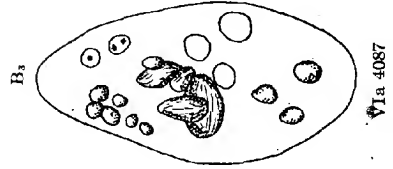
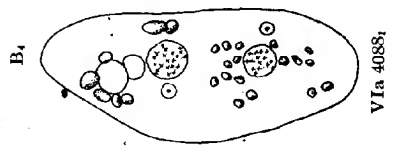
individuality of the macronucleus is destroyed. The animal in the 4105th generation was lost. The cell in the 4107th generation had already formed two macronuclear anlagen and numerous chromatin bodies. One of the latter was still present at the 4110th generation so that in this case the process persisted over about nine generations.

The next critical period began at the 4140th generation. As already mentioned, Line VI made an abortive attempt at the process in the 4094th generation by the extrusion of a single

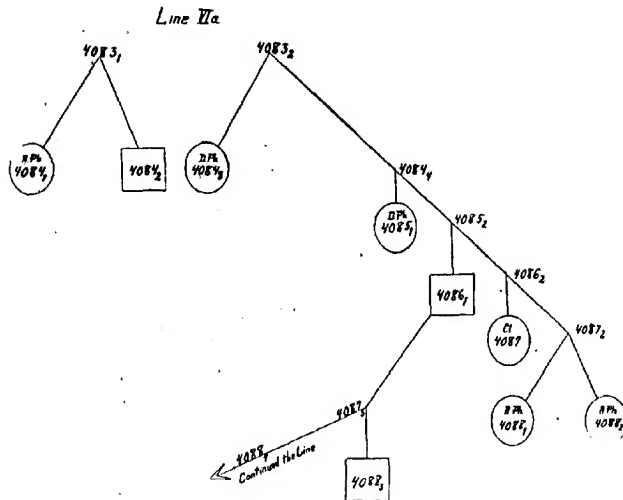


Text fig. 4 Conventional signs, same as in text fig. 1

chromatin body. Line VIa, which was branched from Line VI at the 4068th generation, showed stages of the process from the 4084th to the 4089th generation so that the time of appearance of the process in Line VIa coincides closely with the precocious trial in Line VI at the 4092d generation. In Line VIa the formation of the macronuclear anlagen took place in the 4084th generation (see text fig. 5, B₁). The animal 4084₂ was not preserved but 4084₃ in the descending stage is shown in text figure 5, B₂. Cell 4084₄ continued the line. This cell must have been in the same stage of the reorganization process as 4084₃, because



in the following generations the process is still going on from the 4084th to 4088th generation (Line VIa, text fig. 5, B₁, B₃, B₄, B₅). Therefore, the process does not progress exactly synchronously in sister or cousin cells. This is clear from the diagram, text figure 6. The stages of the process in Line VIa represented in text figure 5 show the climax and the ascending phase (B₁, B₄, B₅). Cell 4087 is about to form the macronuclear anlagen. Their formation took place in cells 4088₁ and 4088₂ (B₄, B₅).



Text fig. 6 Diagram showing a case of nonsynchronism of the reorganization process in closely related cells.

The fact that the sister cells show slightly different stages in the process of course has no significance from the standpoint of the synchronal appearance of the process in sister lines; that the latter occurs is apparent from tables 1, 2 and 3 (pp. 462-463) which give the occurrence of the process in the lines branches from Line VI. Thus in VIa, the process was going on in the 4084th generation, in VIb in the 4092d generation. An examination of table 1 shows that the process was observed in Line

TABLE 1

Showing the occurrence of the reorganization process in Line VI and its derivatives, Culture IE

PERIODS	4020-4050 GEN.	4050-4100 GEN.	4100-4150 GEN.	4150-4200 GEN.	4200-4250 GEN.	4250-4300 GEN.	4300-4350 GEN.	4350-4400 GEN.	REMARK.
VI		4057	4104	4183	4231				Died in process
4020-4231			4140						
VIa		4084							Died
4068-4095									
VIb		4092		4174					Killed in process
4083-4174									
VIc			4101	4177					Killed in process
4099-4178									
VIId				4180					Killed in process
4178-4180									
VIe				4181					Killed in process
4178-4181									
VIIf				4189	4229				Killed in process
4178-4228									
VIg				4184					Killed in process
4180-4186									
VIh					4236		4315	4355	Killed in process
4189-4358									
VIi					4297				Killed in process
4246-4297									
VIj*							4312		Killed in process
4306-4315									
VIk*									Killed during low division rate; animal not in process
4325-4336									
VI*								4355	Killed in process
4348-4355									

* Sub-lines j, k, l were branched from i.

VI in the 4104th generation, in VIc in the 4101st generation. Further evidence of the synchronal occurrence of the process is afforded by the next period in table 1 which shows the process in Line VI at the 4183d generation and in six branches from this line (VIb, VIc, VIId, VIe, VIIf, VIg) between the 4174th and 4184th generation. These facts are substantiated by further data as presented in the graphs of the division rate.

The third repetition of the reorganization process in Line VI took place between the 4140th and 4146th generation. This was relatively early since the second occurrence of the process

TABLE 2

Showing when the reorganization process occurred in Line III and derivatives, Culture IE

PERIODS =	4020-4050 GEN.	4050-4100 GEN.	4100-4150 GEN.	4150-4200 GEN.	4200-4250 GEN.	4250-4300 GEN.	4300-4350 GEN.	4350-4400 GEN.	REMARKS
III 4020-4358		4065		4189	4237		4315		Killed at end of experiments
IIIa 4246-4275						4271			Died after process
IIIb 4304-4321							4313		Killed; animals small
IIIc 4327-4346									Killed; division rate low, not in process

was at the 4104th generation. The ascending phase of the process was completely secured (text fig. 7). Cell C₁ (4140th generation) has two macronuclear anlagen; C₂ (4141st generation), resulting from one division, has one macronuclear anlage; C₃ (4142d generation) shows the distribution of the chromatin bodies in the daughter cells.

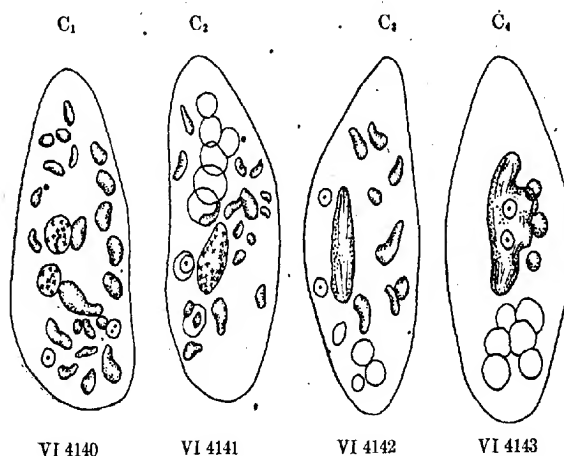
The next occurrence of the process in Line VI, represented in text figure 8, gives nearly a complete picture of each stage in the process in a *single* line. The animal (4180-4181) just dividing was typical, while the following cell (4181-4182) shows simply the extrusion of one chromatin body and the remnant of a

TABLE 3

Showing when the reorganization process occurred in Lines I, II, IV, and V, Culture IE

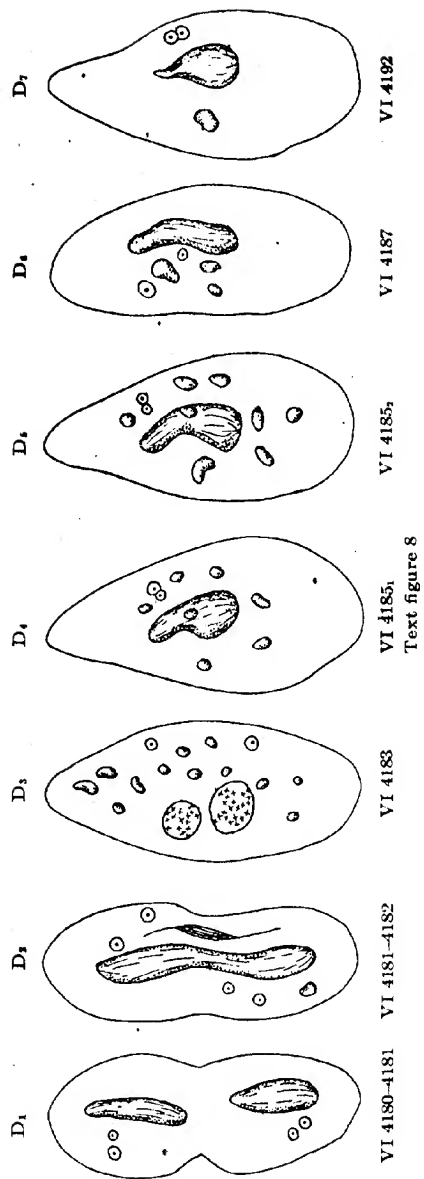
PERIODS =	4020-4050 GEN.	4050-4100 GEN.	4100-4150 GEN.	4150-4200 GEN.	REMARKS
Line I from 4020 to 4037 gen.	4038				Died in process; last animals small due to abnormal division; lived 4 days without division.
Line II from 4020 to 4172.....	4021	4069		4172	Line killed in process
Line IV from 4020 to 4075.....		4066			Animals small; died
Line V from 4020 to 4170.....	4023		4115		Killed; not in process

division spindle. This proves that in generation 4182 the complete extrusion of chromatin bodies will be completed and the 'reduction' divisions of the micronuclei and their degeneration must also occur here during the thirty-six hours that this cell persists as an individual. The low division rate at this point indicates that the profound nuclear changes temporarily inhibit the reproductive activity. The animal undergoing the next division (4182-4183) was not preserved but evidently would have shown each of the arising cells in the climax of the process

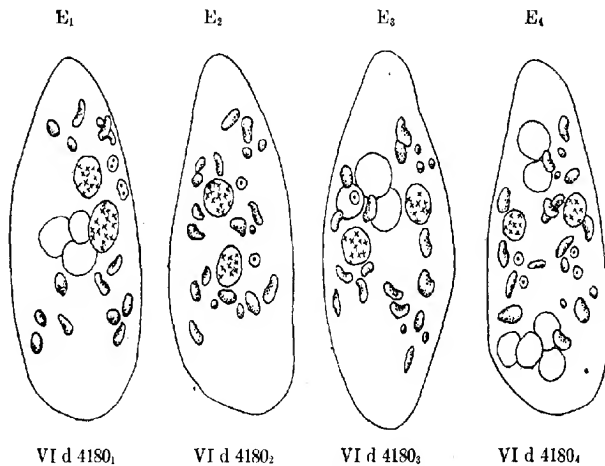


Text figure 7

with one or two micronuclei and numerous chromatin bodies. However, one of the completed products of this division, 4183d generation (D₃), shows the two macronuclear anlagen completely formed. The next stages (4185₁, 4185₂, 4187) show the distribution of the chromatin bodies. Cell 4184 was not preserved but evidently it had one macronuclear anlage, so from generation 4182 to 4192 (D₇) with one chromatin body, we have the process characterized physiologically by the lowering of the division rate in the 4182d to 4183d generations when reproduction was deferred for about thirty-six hours, and then the sudden acceleration



ation of the rate at the 4187th to 4192d generation when three divisions occurred in the twenty-four hours. The morphological characteristics for the single periods are well established as text figure 8 (D_1 to D_7) proves. The descending phase of the process is of the shortest duration and this makes it particularly difficult to secure all the stages—several coming and going in a single generation. On the other hand the ascending phase is the longest and, therefore, the stage most often seen in all lines is that with two macronuclear anlagen. The synchronism of the process in Lines VI, VIb, VIc, VId, VIe, VIf and VIg made it possible

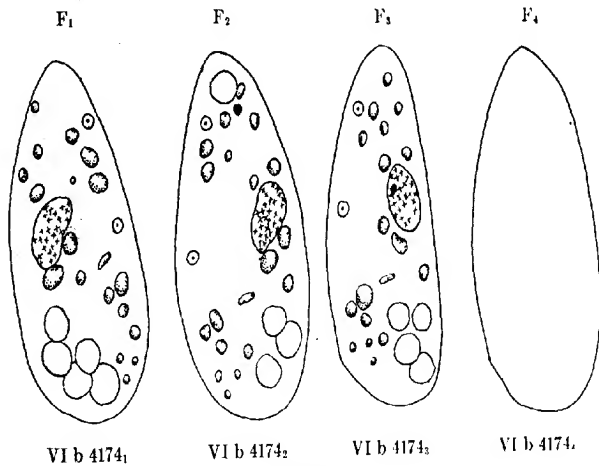


Text figure 9

to kill whole lines undergoing the process and in this way some of the most interesting stages were secured.

Text figure 9 (E_1 to E_4) gives four cells (line VId) all at the same stage with the formation of the two macronuclear anlagen completed. The next stage will be the distribution of the two macronuclear anlagen into two sister cells. This stage is simultaneously completed in four cells of Line VIb at the 4174th generation. Text figure 10 (F_1 to F_4) shows three of these cells

which result from two divisions in forty-eight hours. The fourth animal (F_4) was lost. Since each of the three animals preserved has one macronuclear anlage it is certain that each of the two parent cells in the 4173d generation had two macronuclear anlagen, one of which passed to each daughter cell at division. The fourth animal (F_4) which was lost during preparation obviously must have been in the same stage as those figured. The next text figure, 11 (G_1 to G_3), shows again that at least seven or eight generations are necessary to complete the process. The



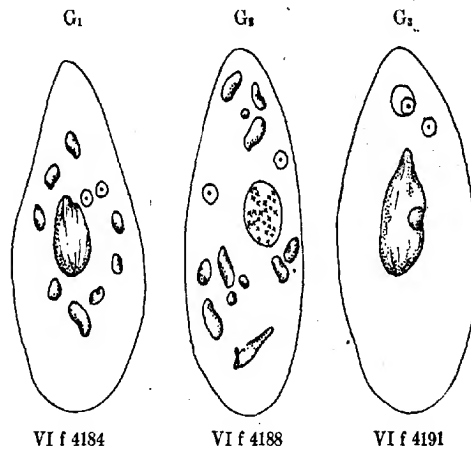
animals are from Line VI \bar{f} in the 4184th, 4188th, and 4191st generation and show the extrusion of the chromatin bodies from the old macronucleus, the new macronuclear anlagen and the nearly reorganized paramaecium cell.

The last occurrence of the process in Line VI extended from the 4229th generation to the death of the line at the 4231st generation from the accidental infection of the culture medium with a deleterious strain of bacteria, as mentioned on pages 478 and 489. Five times in 211 generations the cell reorganized its nuclear

apparatus by the process; six times the division rate (see p. 478) was relatively low. Five times this was synchronous with the process; once with bacterial infection.

The process occurred in Line VI and its sublines at intervals of about forty to fifty generations. For example, Line VI had the process in progress at the 4231st generation, Line VI f at the 4229th generation, and Line VI h at the 4215th generation.

The stages of the process which occurred in Line VII in the 4279th generation and Line VI h in the 4315th and 4355th gen-



Text figure 11

eration, and in Line VI j in the 4312th generation did not show any new features. Simultaneously with Line VI h, Line VII underwent the process in the 4355th generation. Line VI k was killed after eleven generations, when the division rate fell slightly, with the expectation of securing early stages of the process. However the process was not found but judging by closely related lines it would have appeared about twenty generations later. Line VI together with its twelve branches and subbranches exhibited the process a total of twenty-one times from the 4020th to the 4355th generation at clearly recognizable periods, showing that

there is an inherent tendency for sister lines to undergo the process synchronously. The significance of this will be considered later.

The death of Line VI during the process indicates a fact which we have noted several times in these experiments and one to be expected when the complexity of the reorganization process is appreciated. The animals at this time are in a relatively susceptible condition and consequently more readily succumb to slight environmental changes. It is undoubtedly at such periods that many cultures not bred under the most favorable conditions have become exterminated, and the attending cytological conditions have naturally been interpreted as the results of degeneration.

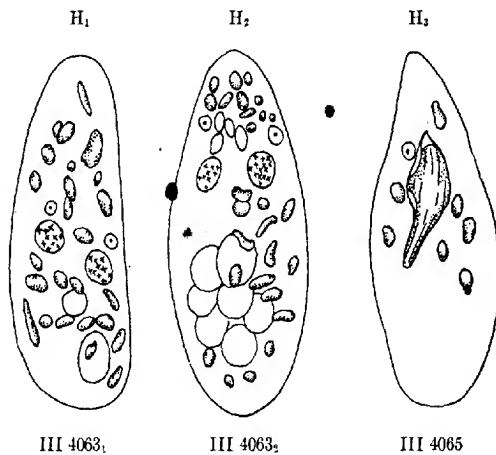
The cells during the process seem to be more opaque than usual, their breadth is relatively greater, their total volume is somewhat increased and their movements are considerably more sluggish. Cells with this appearance defer division for about thirty-six hours and are at the climax of the process. The animals at this time are more difficult to handle with a pipet owing to the fact that they have a tendency to adhere to the glass or to minute particles of debris, thus indicating the so-called miscible state noted by Calkins, Erdmann, Popoff and others in conjugating forms. Also, animals in this condition will sometimes burst on transference to fresh culture fluid medium or to a fixing fluid.

Thus, by the continued daily study of the animals and their rate of division one finally becomes able to tell, with a considerable degree of accuracy, the occurrence of the process merely by the use of a low power of a Zeiss binocular microscope.

B. SERIES OF CELLS FROM LINES I, II, III, IV, V

Line III, which was not under such close observation as line VI, had the process at the 4065th, 4189th, 4237th, 4315th generations. Text figure 17 gives the graph of the division rate averaged for five-day periods. One lowering of the division rate occurred at generations 4140 to 4144. During this time the

cells from Line III were not preserved but judging by the division rate of the line and the occurrence of the process at this period in Line VI, it is almost positive that the process took place in Line III at this time. Cell 4148 was preserved and found to be a typical cell without traces of the process. Text figure 12 (H_1 to H_3) give examples of the ascending phase of the process from Line III. The cells 4063₁ and 4063₂ had formed the macronuclear anlagen. Two divisions later, cell 4065 (H_3) had only seven chromatin bodies, showing a reduction of these bodies by



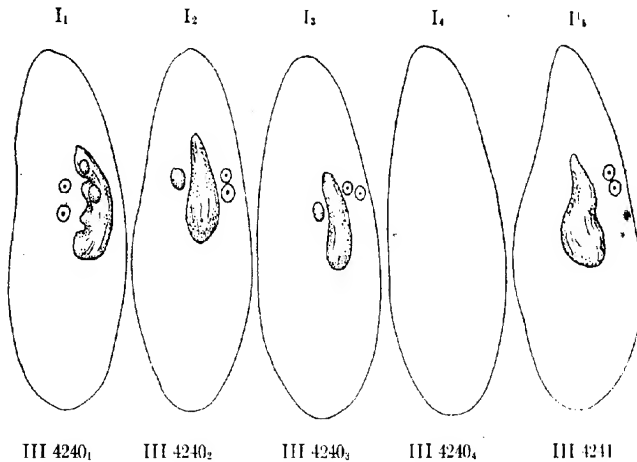
Text figure 12

distribution and absorption from twenty-seven (H_2) to seven during these two cell generations. Text figure 13 (I_1 to I_5) give some of the later stages of the ascending phase. The three animals which could be observed had a few chromatin bodies. Cell 4240₁ had two, 4240₂ one, 4240₃ one chromatin body. Cell 4240₄, which continued the line, gave rise to typical animals at the following division, 4241st generation (I_5).

Text figure 14 (J_1 to J_3) shows the process after the formation of the macronuclear anlagen in cell 4189. After two divisions the new macronucleus is seen in the sister cells 4191₁ and 4191₂.

Thus the distribution of the two macronuclear anlagen took place in the 4190th generation, which affords further proof that *the two macronuclear anlagen are distributed by one cell division to each of the resulting sister cells.*

In Line III and its branches, IIIa, IIIb, IIIc, the process was actually observed six times, while undoubtedly it also occurred several times unobserved, judging by the division rate and the periodic occurrence of the process in sister lines (see table 2 and text fig. 17).

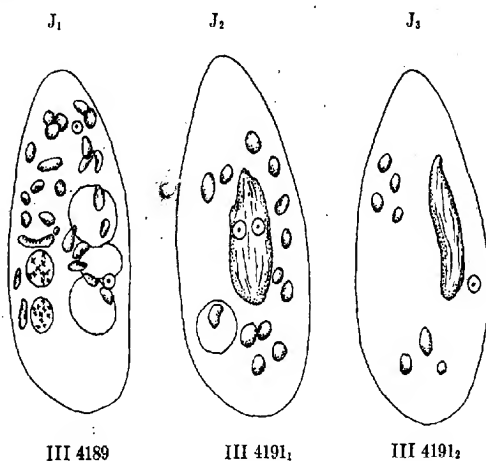


Text figure 13

The Lines I, II, IV, V, which were not branched, show the process a total of seven times, each time at due periods coinciding with Line VI. Some of the stages observed in these lines are presented in the plates as they supplied some of the details of the process as, for example, Line II, 4069th generation (fig. 9, pl. 1) which give a particularly clear idea of the extrusion of the chromatin bodies.

One point which is shown by a study of tables 1, 2, and 3, which give the life history of the twenty-one lines, is the occurrence of death in certain instances during the process. Of these

lines ~~seventeen~~ were killed intentionally, while of the remaining four lines, three died during or shortly after the process. Some of these animals showed irregular divisions resulting in very small cells which apparently lacked the power of growth. It is important here to emphasize that fatalities may occur as a result of faulty reorganization during the process just as has so often been observed to occur in conjugating animals. Both conjugation and the reorganization process, however, are normal phenomena which have no pathological features.



Text figure 14

The detailed discussion of Lines I to VI and their branches and subbranches gives clear evidence that the process, resulting in a reorganization of the nuclear apparatus which simulates typical conjugation, is going on with strictly determined morphological and physiological features at relatively definite periodic intervals of time in the pedigreed race of *Paramaecium aurelia* which is the basis of this investigation.

V. THE REORGANIZATION PROCESS IN THIS RACE AFTER CONJUGATION AND IN OTHER RACES AND SPECIES OF PARAMAECIUM

So far it is clear that the nuclear reorganization occurred throughout the life of this race bred under conditions which absolutely precluded the occurrence of conjugation, and the question arises here: Would the process again occur periodically after conjugation had taken place? Animals were accordingly isolated from this race at the 4102d generation and with them a large mass culture was started in a slender dish. Within a few days a number of conjugating pairs were observed and about twenty were isolated. Some of the animals were preserved for details of conjugation in this race, while the descendants of other ex-conjugants were bred on depression slides by the same isolation culture methods as those used in the main culture from which they were derived at the 4102d generation. A study of the animals preserved from these ex-conjugant lines demonstrates that *the same reorganization process was resumed in all the lines within a relatively short time after conjugation.*

More positive evidence could hardly be presented to prove that the process is a fundamental normal periodic phenomenon in the life of this race of *Paramaecium aurelia*.

This being established, the question arises: Is this a peculiarity of this race or does the process occur generally in *Paramaecium aurelia*? This we can also answer conclusively. Erdmann on August 11, 1912, isolated a specimen of *Paramaecium aurelia* from a canal of the river Spree in Berlin and bred its descendants by the daily isolation method on a culture medium of beef extract. Specimens from Erdmann's race, bred by this method which absolutely prevents conjugation, were preserved from time to time and figure 24 (pl. 2) shows one of these in a characteristic stage of the process under discussion, i.e., the formation of the macronuclear anlagen. Thus it is evident that the same nuclear reorganization which has occurred throughout the life of Woodruff's race started with a specimen of *Paramaecium aurelia* isolated at random in America in 1907 also occurred, in Erdmann's race similarly isolated in Germany in 1912.

Therefore the data justify the conclusion that this reorganization process is a normal phenomenon and probably occurs in all races of the species *Paramaecium aurelia*.

Most suggestive observations on nuclear changes in *Paramaecium aurelia* were made incidentally by Hertwig during his study of conjugation in this species. This author noted ('89, pp. 74-75) in a mass culture, in which conjugation had not been observed for a long period, certain animals whose nuclear structure apparently indicates isolated steps in the phenomenon which are described in detail in the present paper, and the significance of which is here elucidated.

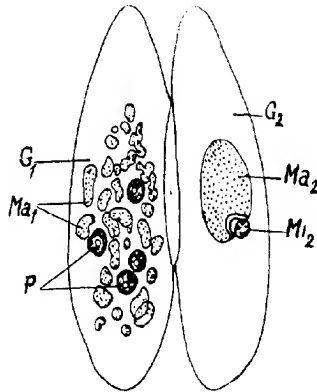
Hertwig grouped the stages which he observed under two general classes, one of which he interpreted as merely a vegetative phenomenon, in which a *new* nuclear apparatus is not formed:

Die zuerst eintretenden Veränderungen besitzen kein Analogon in den Vorgängen einer normalen Entwicklung. Wahrscheinlich zerfällt der Hauptkern erst in grössere dann in kleinere Stücke, ohne das regelmässige Auswachsen in Fortsätze, welches im Lauf der geschlechtlichen Entwicklung der *Paramaecium* eintritt. Ich fand bald 2, bald 4 Nebenkernkerne entweder in Form der ruhenden Kerne oder häufiger in Form von Spindeln, wie ich sie ebenfalls sonst nicht beobachtet habe.

Our data show this to be a mistake. These stages are clearly some steps in the descending phase of the process just before the beginning of the climax.

The second group of changes of the nuclear apparatus described by Hertwig comprised: "Tiere mit vergrösserten Nebenkernen, mit Sichelkernen mit 2, 4 und 8 Spindeln, Tiere, bei denen die Teilung in die Haupt- und Nebenkernanlagen vollzogen war."

From this brief note it is clear that the animals with two and four spindles may possibly represent ordinary vegetative division because the striking disintegration of the macronucleus is not mentioned. We have never discovered an animal with *eight spindles*, but "Tiere, bei denen die Teilung in die Haupt- und Nebenkernanlagen vollzogen war" are apparently identical with those animals which we figure (figs. 21, 22, pl. 2) and represent the important reconstruction of the new nuclear apparatus. If it be true that in Hertwig's culture conjugation had not oc-



Text figure 15

current (it is impossible to prove this since he employed mass cultures in the work) then it is also true that he discovered at least one of the steps in the process which is outlined in the present paper but obviously he failed to recognize its general fundamental importance. This is evident from the trend of his work and that of his students, on the life history of Infusoria, during the subsequent quarter of a century.

Another question naturally suggests itself at this point: Is the process confined to *Paramecium aurelia*? Figure 34 (pl. 3) is from a pedigreed race of *Paramecium caudatum* which we have studied by the same experimental methods, and shows a characteristic stage in the process. This indicates that it occurs, at least with essentially similar features, in *Paramecium caudatum* also. It will be shown in a later section of this paper that certain of the morphological changes interpreted by Calkins, Popoff and others as degeneration phenomena in *Paramecium caudatum* are in all probability the same process. It may be mentioned here, however, that Doflein ('07) figures an abnormal conjugating pair of *Paramecium caudatum*, one animal of which appears to be a typical vegetative animal while the other shows what he interprets as conjugation nuclear phenomena in a single

animal. From our results we would interpret this as a stage in the ascending phase of the process, because he figures the four macronuclear anlagen typical for *Paramaccium caudatum* and terms them Mikronukleusderivate (see text figure 15, which is a copy of Doflein's figure).

Finally, does the process occur in other genera of Infusoria, and in other classes of Protozoa? Later in this paper we shall discuss the literature relative to this point but it can be stated here that while no positive evidence is extant to answer this question, because pedigreed cultures involving a daily detailed cytological study have not been made, nevertheless, such more or less random facts as may be gleaned from various investigations strongly suggest, at least, that the authors are dealing with comparable nuclear reorganization phenomena.

VI. THE REORGANIZATION PROCESS AND ITS RELATION TO RHYTHMS

Earlier work on this culture has shown that there are rhythms in its rate of reproduction which are not the results of environmental variations, but which are due to some periodic internal phenomena of unknown character.

Minor fluctuations are evident in the division rate of other species of Infusoria bred by the daily isolation method first employed by Calkins. The fluctuations in the culture graphs of Calkins on *Paramaecium caudatum*, Woodruff on *Oxytricha fallax* and *Gastrostyla steinii*, Gregory on *Tillina magna*, and Moody on *Spathidium spathula* appear to be of the same character as those demonstrated in this culture. However, since the authors in the study of the life history of these species have not eliminated the possibility that the fluctuations in the division rate are the results of environmental changes, it is impossible to state positively that the fluctuations observed by them are actually 'rhythms,' though it is highly probable that such is the case.

It is important to emphasize the fact, although it is well known, that relatively slight changes in culture medium or tem-

perature will change the division rate. Indeed it may be said that the rate of reproduction is a function of the environment of the cell—except as the rhythms interfere—and consequently the fluctuations which appear in the graph of the division rate of a culture are not, *ipso facto*, true rhythms, i.e., due to “inherent rhythmical changes in the phenomena of the cell” (Woodruff and Bajtsell '11 b, p. 357).

The present cytological study demonstrates the nature of the inherent changes in the cell which have their obvious physiological expression in the rhythms of the rate of reproduction.

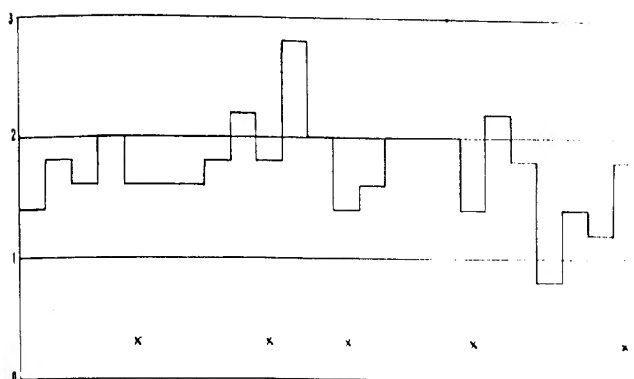
The relations of the process to these fluctuations can be most readily appreciated by a consideration of graphs of the rate of divisions of Lines VI and III of Sub-culture II, which was subjected to the constant culture medium of beef extract and to practically constant temperature conditions (about 26°C.) within the optimum zone for this race.

The five-day period was adopted in the presentation of our results because this was the method of constructing the graph emphasized in the original study of rhythms in this culture. It is realized, of course, that a five-day period is largely an arbitrary one and that the ideal graph would present the momentary changes in the metabolism of the cell. Data for such a curve being absolutely impossible to secure, it might seem at first glance that the daily record of division would approach most nearly to this ideal condition. As a matter of fact, the twenty-four-hour period is as arbitrary as the five-day period when it is considered that this is a long period when compared with the metabolic changes in the cell and that the daily record, made at approximately 11 A.M., would merely give the divisions actually *completed* during the previous twenty-four hours. For example, let us assume that, at the time of isolation, two animals are present, representing one division during the previous twenty-four hours. The record for that day is one division. One animal is then isolated and it divides within an hour and each of the resulting cells again divide twice before the next isolation. The record for this second day is three divisions, thus the record for the two days shows a different division rate for each day, i.e.,

one division against three divisions, whereas a more true, but not a perfect, picture of the state of affairs is given by the statement that four divisions occurred in forty-eight hours. One might follow this argument to its logical conclusion and assume that the best method of presentation would be to average for considerable periods, e.g., 10 or 30 days, but this obviously would tend to obliterate any fluctuations in the rate which are not of relatively long duration. The adoption of the five-day period was made in recognition of both of these contingencies, and it was of a duration particularly well suited to show the effect of the process on the reproductive rate, because the process extends over about nine cell divisions or a period of about six days. Consequently the effect of the process makes itself evident in the five-day plot. Certain apparent irregularities in the coincidence of the phenomena are, from an actual study of all the data at hand, clearly due to the fact that the five-day period is not ideal.

In a consideration of the relation of rhythms to the reorganization process it will be convenient to consider first the data from Line VI, Subculture IF, since animals from this line were preserved every day of its existence and, during the process, every animal was preserved except the one needed to keep the line alive.

Inspection of the graph of this line shows at a glance five fluctuations in the reproduction rate which would naturally be interpreted as rhythms (cf. text fig. 16) while a study of the cytological condition of the specimens shows that the low point of each of the first four fluctuations is coincident with the profound nuclear changes of the reorganization process. The fifth fluctuation, during which the division rate fell to nearly three-quarters of a division per day, was brought about by the contamination of the medium at the time of transference with a deleterious strain of bacteria, from the effects of which the animals of this line never completely recuperated and succumbed upon the recurrence of the process for the fifth time. Consequently it is evident that four out of the five fluctuations are

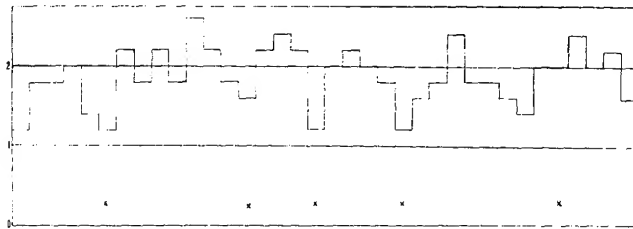


Text fig. 16 Graph of the rate of division of line VI, subculture 1E, averaged for five-day periods. The periods during which the reorganization process occurred are indicated by an X.

actually rhythms, i.e., not obviously due to changes in the environment, and at the low point of each of these rhythms the process was in progress. Table 1 shows that the process in Line VI was in progress at the 4057th, 4104th, 4140th, 4183d, and 4231st generation when the line died. These generations represent approximately the climax of the process. Thus it is clear that in the line under consideration the process occurred at intervals of about 40 to 50 generations or about every twenty-five to thirty days, and that the low point between each of the first four fluctuations was coincident with the nuclear reorganization. This evidence from Line VI shows clearly a relationship between the rhythms and the nuclear reorganization.

Cumulative evidence which establishes a casual relationship between rhythms and the process is afforded by a study of Line III which was carried continuously for eight months. Animals from Line III were not preserved every day at the time of isolation (as was the case with Line VI) owing to the great amount of labor involved, but at intervals of several days duration. That is, no attempt was made to have a complete pedigreed series of stages showing its daily cytological condition but merely a broad

view of its nuclear state. By this method the process was discovered at the 4065th, 4189th, 4237th, 4315th generation (cf. table 2). Text figure 17 gives the graph of the division rate of this line, plotted the same as that already presented for Line VI, and shows the periods in which the generations involving nuclear reorganization were discovered. Here again in four out of five cases, the process coincides with the low point in division rate, i.e., between two rhythms. The occurrence of the process of the 4315th generation, however, coincides with the early ascending phase of the rhythm and is an exception for which the data afford no evident explanation.



Text fig. 17 Graph of the rate of division of Line III, subculture IF, averaged for five-day periods. The periods during which the reorganization process occurred are indicated by an x.

The other main lines were carried chiefly for the purpose of affording a sufficient supply of animals in the process and, after the first couple of months, were preserved only when the process was suspected from the appearance of the cells, the rate of division and the length of time and the number of generations since its last occurrence. Consequently it is unnecessary to consider these lines in detail from the standpoint of the rhythms but simply to emphasize that the evidence derived from them is entirely corroborative of that presented from Lines VI and III (cf. table 3). In our description of the cytological changes in the process it has been shown that the phenomenon extends over about nine cell generations, and that at the climax division is deferred for a period of nearly 36 hours. *Therefore, it is*

evident not only that the reorganization process is coincident with the low point between two rhythms, but also that there is a causal relation between the reorganization process and the rhythms.

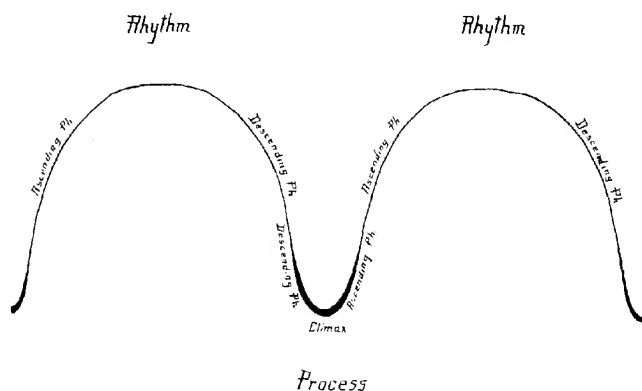
VII. THE REORGANIZATION PROCESS AND ITS RELATION TO DEPRESSION PERIODS

The results of the great majority of studies on various species of Infusoria, from the pioneer experiments of Bütschli and Engelmann, have indicated that these forms will not reproduce indefinitely if conjugation between two animals is prevented, but that the race shows signs of lowered vitality accompanied by marked morphological changes which sooner or later end in death. Improved methods of conducting the cultures, however, have enabled succeeding investigators to continue the races longer and longer, until studies on the culture which forms the basis of this paper have shown that under proper environmental conditions *Paramecium aurelia* can be bred indefinitely without conjugation or artificial stimulation. In other words, there is no evidence of a life cycle, as understood and emphasized by Maupas, Calkins and other authors, which comprises a more or less definite number of cell generations beginning after conjugation with the high potential of vitality of youth and maturity and leading to either old age and death or conjugation and rejuvenescence again.

A critical survey of the literature, however, shows that another type of variation occurs in the life history of Infusoria under culture conditions which must be distinguished from the cycle. In certain cases this has been recognized by the author, but as a rule it has been passed over without comment owing to the fact that only by the daily isolation method of culture is it possible to demonstrate its reality. Apparently Hertwig ('00-'04) from his study by mass culture methods of the life history of *Actinosphaerium eichhornii*, *Dileptus gigas* and *Paramecium caudatum* was the first to contrast minor periods and deep periods of physiological depression, the latter resulting in death. The lesser periods were marked chiefly by a slight lowering of

the general physiological activities of the cells, and interpreted as the result of relatively slight disturbances of the nuclear condition which were overcome by internal readjustment. In the periods of deep depression, on the other hand, marked signs of degeneration were evident, for which the sole panacea was conjugation. This led Hertwig to the natural suggestion that the nuclear phenomena observed in physiological depression and those which occur at conjugation have fundamentally the same *raison d'être*.

Calkins ('04, p. 424) in his studies on *Paramecium caudatum* noted that the well-marked cycles which resulted in death, unless



Text fig. 18 Diagram illustrating the relation of the reorganization process to rhythms.

drastic methods of artificial stimulation were resorted to, were of about six months duration, while intermediate cycles of less importance occurred at intervals of approximately ninety days, recovery from which took place without purposeful stimulation. In these smaller cycles morphological signs of degeneration were not observed, but in the well-marked cycles cytoplasmic and nuclear changes occurred. These varied somewhat in character at the low points of the various cycles, but in several instances Calkins was able to restore the normal condition by the oppor-

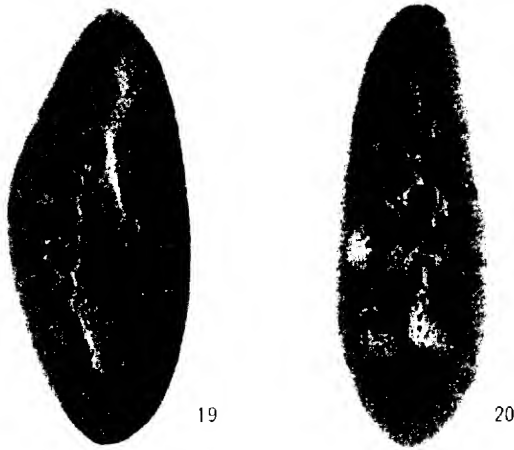
tune use of artificial stimuli. At the last cycle when all the methods of rejuvenation which were tried proved of no avail Calkins observed that the signs of degeneration were apparent in the micronuclei and, therefore, concluded that at last 'germinal death' occurred.

Some very suggestive nuclear conditions are figured by Calkins ('04) from animals in his depression periods. Calkins' figure 15, plate 2, shows an animal of his B series just before its death in the 502d generation, which had been treated with beef extract. He states "that the micronucleus has divided three or more times and the daughter nuclei have accumulated at one end." His photograph does not convince us that these are really micronuclei. If they are they clearly represent an early "reduction" phase of the process as described by us for *Paramecium aurelia*. We are inclined, however, to interpret these 'micronuclei' as chromatin bodies since we have found that in *Paramecium caudatum*, as in *Paramecium aurelia*, this is the method of dissolution of the macronucleus during the process. This figure of Calkins should be compared with our figure 34 (pl. 3) which shows the chromatin bodies in *Paramecium caudatum*. Text figure 19 (Calkins' fig. 16, pl. 2) shows an animal from his

A series in the 602nd generation treated for twenty-five minutes with phosphoric acid. It was transferred to hay infusion and killed twenty-four hours afterwards. The macronucleus is broken into fragments; the micronucleus has divided and one part (left center) seems to be forming a new macronucleus. (This individual offers the only evidence obtained of nuclear fragmentation and reconstruction through artificial means.)

This suggestive comment by Calkins, given merely in his description of plates, apparently hits the mark. We must interpret this depression animal of Calkins, which he attempted to 'rejuvenate' by phosphoric acid, as at the end of the climax of our process with many chromatin bodies and two macronuclear anlagen. The micronuclear condition is not clear from the photograph (compare text figs. 19 and 20, p. 484).

Maupas' observation that in certain hypotrichous forms the micronuclei in some periods may be increased to a number



Text figures 19 and 20

beyond that typical for the species was substantiated by Woodruff ('05) who observed marked macronuclear fragmentation as well as a tendency to micronuclear reduplication at periods of low division rate in a culture of *Oxytricha fallax*. The fact that Woodruff was able to 'rejuvenate' the race when apparently on the verge of extinction led him to state that "we are hardly justified in assuming that Protozoa, when dividing at a low rate, with nuclei fragmented, etc., are exactly 'abnormal,'" and "suggests that we are justified in regarding these changes as phases in the life history of Infusoria which occur under certain conditions after a considerable period of vegetative reproduction." It now seems probable that this tentative suggestion points in the right direction. The discovery of atypical conditions, chiefly during the period of deepest depression and throughout the month that it persisted before 'rejuvenation' occurred, would seem to indicate that owing to unfavorable environmental conditions the race of *Oxytricha* was in an unhealthy state which resulted in a series of abortive attempts to reorganize itself, the final one of which was successful.

Popoff ('07) found in the culture of *Stylonychia mytilus*, which he bred for three and one-half months, that after three periods of depression had occurred the race finally became extinct during a period of "sehr tiefe Depression." Popoff does not make a distinction between these various depressions, except in regard to their intensity. He records the fact that at these times characteristic nuclear changes as well as a tendency to conjugation were in evidence though both were most pronounced during the very deep depression. Again, in a culture of *Paramecium caudatum*, the data from which he does not present in as much detail, he found essentially similar depressions and nuclear metamorphoses. He identifies the deepest periods of depression with those described by Maupas as "dégénérescence sénile" by Hertwig as "physiologischer Tod," and by Calkins as "depression periods."

In a later study Popoff ('09) stated that he was able to produce, by treatment with various chemical reagents, identical variations in the nuclear apparatus of cultures of *Stylonychia mytilus* and *Paramecium caudatum*, which in turn he considered similar to those observed by Kasanzeff in starved Infusoria. In other words, Popoff concluded that the nuclear phenomena in all his depression periods are exactly the same as those induced by starvation, chemical stimuli, etc., and further that they cannot be distinguished from those which occur at the onset of normal conjugation. In text figure 21 (Popoff's fig. 25, pl. 2) is shown an animal with two micronuclei in mitosis from a culture of *Paramecium caudatum* which Popoff had treated with ammonium. This specimen we would interpret as an early 'reduction' division in the reorganization process under discussion.

We have found that it is possible to retard or hasten the occurrence of the process by the character of the culture medium: for example, it may occur a few days earlier in animals not supplied daily with fresh culture fluid than in the regular lines. Consequently we can readily believe that treatment with the reagents employed by Popoff would influence its onset. Popoff figures specimens of *Stylonychia mytilus* in periods of depression and also after chemical treatment which he interprets as showing



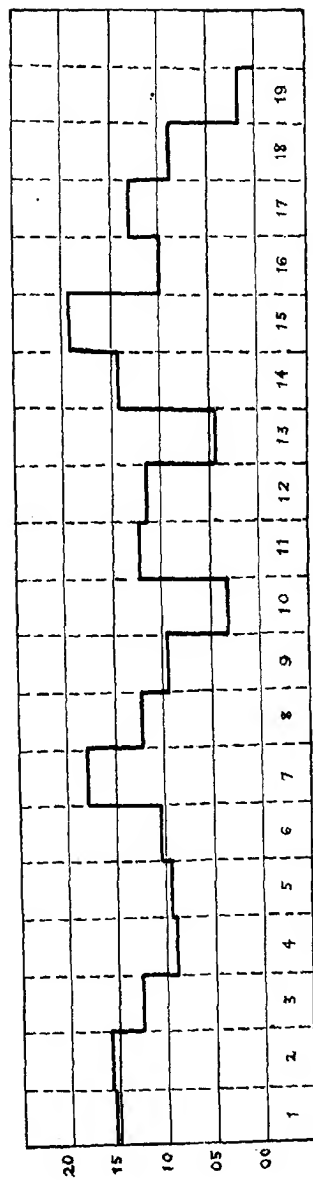
Text figure 21

the multiplication of micronuclei until there are a dozen or more in a cell. Although it seems clear that these represent stages in the process, it is impossible from his figures to be sure that many of the bodies which he interprets as micronuclei are not chromatin bodies which have arisen from the macronucleus as occurs in *Paramecium aurelia*. However, Popoff's paper shows that he recognizes a general similarity of the so-called depression phenomena and the cytological changes incident to the early stages of conjugation. Our data, secured by a daily cytological study of pedigreed animals, throw light upon Popoff's isolated stages and indicate with considerable certainty that they are stages in the sequence of normal nuclear changes in the process which closely parallel conjugation both in morphological and physiological features.

Gregory ('09) in a study of the life history of *Tillina magna* points out that " . . . the curve which represents the general vitality of the protoplasm shows the normal rhythmic fluctuations observed by Woodruff," and in an analysis of the

data secured by Popoff in his study of the life history of *Stylonychia mytilus* shows that "if the curve of *Stylonychia* is plotted from average records of five and ten-day periods, it will be found to correspond to the curves for *Paramecium*, *Oxytricha* and *Tillina*, each showing the rhythmic periods of high and low vitality." We reproduce here (text fig. 22) Gregory's curve plotted from Popoff's data, with the depression periods indicated. This curve is obviously strikingly similar to the ones which we give in the present paper to show the relation between the process and the rhythms. It is to be noted that at the low points between Popoff's 'rhythms,' just as at the low points between our rhythms, nuclear changes occurred. This agreement, taken in conjunction with the fact that Popoff's animals show evidence of the process we describe, probably makes it now safe to state that Popoff's small depression periods are rhythms. Such being the case, we are in a position to identify with more certainty the **minor depression periods of Hertwig and Calkins as rhythms also.**

When one considers the diverse culture methods used by the various authors and that merely cells selected at relatively long intervals and practically at random were studied by them, it is remarkable that so many indications of the process may be gleaned from their data. This affords additional evidence of the general occurrence of this phenomenon in Infusoria. We do not desire, however, to deny that abnormal cytoplasmic and nuclear conditions can and do occur in infusorian cultures, or to seem to attempt to interpret as stages of the process all the so-called degeneration stages figured by various authors. Now and then an animal in the culture of *Paramecium* under consideration has failed to divide for several days and has finally died without signs of the process. A study of such animals has shown what are certainly abnormal conditions of cytoplasm and nucleus. The cytoplasm appears "diluted" and vacuolated, the pellicle has a characteristic glistening effect, and the cell-contents show more acidity than normal. The nucleus may assume various atypical forms. Such a condition may arise in various ways, but the usual one is the contamination of the medium with a



Text fig. 22 Complete history of Stylonychia. The curve is plotted from the records of Popoff. Rate of division averaged for five-day periods. Periods 4, 10, 13, 16 are the "deep depression" periods of Popoff. (From Gregory, 1909.)

deleterious bacterial strain. Some types of bacteria produce unhealthy conditions which are readily recognizable when one has conducted a culture for a long time and consequently, when such appear, they can be eliminated. Now and then, however, a culture becomes contaminated with a form which does not produce a characteristic change in the medium and therefore it is not noticed. As a result, at the daily isolations, the fresh medium becomes infected. This was the case with Line VI of Sub-culture IE, as already described (cf. p. 467). The Infusoria, then, can and do degenerate and die as a result of unfavorable conditions, but we wish to reiterate that even under such conditions the cell sometimes attempts to restore its normal equilibrium by the inherent reorganization phenomenon.

It is clear that the cycle emphasized by Maupas, Calkins and others is merely a phantom which has continually receded as each successive investigator has approached the problem with improved culture methods until it has vanished with Woodruff's race of (so far) 4500 generations. What remains then is the rhythm and in the light of the present study, which demonstrates the underlying cytological phenomena of which it is an outward physiological expression, the whole problem takes on a new aspect. The cell automatically reorganizes itself periodically by a process which, in its main features, simulates conjugation—but without a contribution of nuclear material from another cell. Therefore it is evident (as has been shown by this culture) that the formation of a syncaryon, whose components are derived from two cells, is not necessary for the continued life of the cell—it has an internal regulating phenomenon which is entirely adequate to keep it indefinitely in a perfectly normal condition.

VIII. ENDOMIXIS AND ITS RELATION TO CONJUGATION

It is necessary now to consider the significance of the reorganization process in relation to conjugation in which the formation of a syncaryon results in a union of chromatin from two distinct animals.

It has been shown in the main pedigreed culture of *Paramaecium aurelia* that at intervals throughout the seven years (to date) of its existence the process has occurred. It has also been shown that when a mass culture of animals from this race was under just the proper conditions, typical conjugation occurred. *Thus it is proved that both the reorganization process and conjugation are potentialities of the animals of the same race*—and therefore there is no evidence for the view of Calkins ('13) that conjugating and non-conjugating races of *Paramaecium* exist (cf. p. 429), or that "apparently some paramaecia are potential germ cells, others are not." The cell clearly has two methods of reorganization which, so far as the evidence at hand indicates, may be employed indiscriminately. One is conjugation, the other is the reorganization process.

The chief morphological changes incident to conjugation and the process may be contrasted as follows:

In each case the old macronucleus is dissolved in the cytoplasm and micronuclear reduplication occurs until eight are present in the cell;

In conjugation one of these micronuclei persists and by an *extra division* forms the stationary and migratory nucleus. This division is directly preparatory for the accession of foreign chromatin. After the mutual interchange of the reduced micronuclei is effected, a syncaryon is formed. The latter gives rise to the new nuclear apparatus;

In the reorganization process, on the other hand, one or two micronuclei persist (cf. p. 446), from which the new nuclear apparatus arises.

After conjugation the reorganized cell has a new macronuclear and micronuclear apparatus composed of combined material from the two conjugants;

After the reorganization process the reorganized cell has a new macronuclear and micronuclear apparatus composed of material from its own micronuclei.

In a word—the *essential morphological difference is the entire absence of the introduction of foreign nuclear (and cytoplasmic) material into the cell during the reorganization process.*

Since the process results in the dissemination of the material from the old macronucleus and the so-called reduction micronuclei in the cell, it gives the opportunity for a rearrangement of the molecular constitution of the cell. This involves a more profound intermingling of nuclear and cytoplasmic substances than is possible during the typical vegetative life of the cell. *Since this intermingling occurs within a cell we term this reorganization process endomixis.* Endomixis is followed by a slight acceleration of cell phenomena and a new rhythm is initiated.

Similarly, since the consummation of conjugation also results in the dissemination of the old macronucleus and of the 'reduction' micronuclei in the cell it affords the opportunity for a rearrangement of the chemical constituents of the cell. But, further, the exconjugant is supplied with a new nuclear apparatus presumably derived equally from each conjugant. Thus here the introduction of foreign chromatin and possibly cytoplasmic material affords still greater opportunity for chemical rearrangement. Therefore conjugation is a caryomictic phenomenon whereas endomixis, characterized by the non-formation of a syncaryon, is an apocaryomictic process.

The general physiological effect, however, of conjugation in Infusoria is not demonstrated conclusively, though Calkins offers considerable evidence which indicates that, after its consummation, all the processes of the cell, including reproduction, proceed with greater vigor, thus substantiating the view of Bütschli, Maupas, Hertwig and others. Jennings' results on the other hand afford no support for this conclusion.

Without considering this debated question, however, it is evident from this culture that syngamy in Paramaecium is not necessary, under proper environmental conditions, for the continued life of the race, and therefore that the formation of a syncaryon, composed of material partly derived from another cell, is not a *sine qua non* for a proper continuation of all the vital activities of the cell. We would therefore put emphasis on molecular rearrangement as the result common both to endomixis and to conjugation. Since accelerated vital activities follow this chemical change afforded by endomixis, it seems clear

that a similar condition follows conjugation, and therefore that one aspect of conjugation (though it is unnecessary for the life of the race under proper environmental conditions) is dynamic.

Certain observations which we have made on mass cultures of *Paramecium aurelia* indicate that endomixis and conjugation may occur simultaneously in different animals of the same culture, thus strongly suggesting that the same *general* conditions lead to both phenomena—one animal meeting the conditions one way and another by the other, and that both phenomena fill essentially the same place in the economy of life of *Paramecium aurelia*. Therefore it is evident that the vegetative aspect of the life history of *Paramecium* is periodically interrupted by periods in which a dynamic reorganization occurs either by endomixis or by conjugation.

IX. ENDOMIXIS AND ITS RELATION TO PARTHENOGENESIS

One might at first glance consider endomixis as a case of parthenogenesis, owing to certain similarities of the two phenomena. In parthenogenesis the new individual arises from one nucleus which is not a syncaryon, i.e., the fusion of completely independent nuclei. In endomixis the new nuclear apparatus is of micronuclear origin without the formation of a syncaryon. Both parthenogenesis and endomixis therefore are apocaryomictic phenomena which must be contrasted with caryomictic phenomena such as conjugation, copulation and, to a certain extent, autogamy. The introduction of chromatin belonging either to another individual or to another nucleus of the same individual gives an opportunity for an extensive rearrangement of the nuclear substance which cannot be effected to such a high degree in either parthenogenesis or endomixis.

Although both parthenogenesis and endomixis are followed by *Entwicklungserregung* they afford some features which are not identical. In parthenogenesis there is a chromatin reduction which occurs and is compensated for either in the egg itself or in some later period of the life cycle of the race.

Following the definition of Winkler ('08, p. 303), parthenogenesis may be considered as the "apomiktische Entstehung eines Sporophyten aus einem Ei," it being of minor importance whether the reduced or unreduced number of chromosome is present. Strasburger ('07, p. 170) on the other hand puts the greatest weight on the chromosome number and applies the term parthenogenesis only to cases in which an egg with the haploid number of chromosomes undergoes *Entwicklungserregung*. According to the view of Strasburger, the reorganization process in *Paramaecium* cannot be termed parthenogenesis. In endomixis there is no evidence of a chromosome reduction. This race has existed normally without apparent change for over seven years, during which time it has undoubtedly undergone endomixis nearly one hundred times and thus it is clear that a chromatin reduction, involving a loss of specific chromatin or chromosomes and therefore according to the prevailing view a loss of hereditary units or gene material, cannot have occurred in endomixis. Further, the nuclear material from which the new nuclear apparatus arises in endomixis cannot be considered as a gamete, because the third micronuclear division which forms the two gametes in *Paramaecium aurelia* does not occur in endomixis. The eight "reduction" micronuclei are comparable to gametocytes, the paramaecium individual being a gamont before it starts to form the eight so-called reduction micronuclei (Lühe, Hartmann). So according to the definition of Winkler it seems impossible to consider endomixis as parthenogenesis in *sensu stricto*.

We therefore have employed a new term 'endomixis' for the reorganization process in *Paramaecium*, in preference to parthenogenesis which Hertwig applied when he incidentally noted some isolated stages of the nuclear phenomena which we have elucidated. The stages observed by Hertwig were insufficient to give a clue to the sequence of cytological changes—this being necessary for a realization of the importance and proper classification of the reorganization process.

If subsequent research should show (which from our work seems highly improbable) that a third micronuclear division, involving a chromosome reduction, does occur in the descending

phase of the process which we term endomixis, it is obvious that the phenomenon would still fall under endomixis and not under parthenogenesis since the latter term is, according to our interpretation, restricted to cases in which gametes undergo *Entwicklungserregung*. The only way to justify the application of the term parthenogenesis is *sensu stricto* to the phenomenon which we call endomixis would be by proving that in the latter process a reduction of the number of chromosomes actually takes place in a micronucleus which can be considered as the equivalent of a gamete, and that for this reduction there is a compensation.

However, if one is inclined to employ the term parthenogenesis in a very broad sense to include all cases of *Entwicklerregung* without regard either to the character of the cell (egg, gametocyte, gamont, somatic cell) in which they occur, or to haploid or diploid condition of the chromosomes, then endomixis is a new type of parthenogenesis. It is always difficult to relate properly a newly discovered phenomenon to analogous processes. In the present instance this is doubly difficult owing to the fact that in the protista the formation of a typical macrogamete closely analogous with that of metazoa and metaphyta is the exception rather than the rule. It is probable that future study of other *Protista* (e.g., bacteria, amoeba, trypanosomes, plasmodia, etc.) will reveal nuclear reorganization processes which will make certain the proper place of endomixis among the already complicated apocaryomictic phenomena.

X. ENDOMIXIS AND ITS RELATIONS TO VARIATION, HEREDITY AND THE SIGNIFICANCE OF CONJUGATION

We deem it inadvisable in the present paper—which is essentially a description of cytological and physiological observations—to enter into an extended discussion of the wide theoretical bearings of endomixis on the problems of variation, heredity and amphimixis. It is important, however, to consider briefly certain points which are obviously suggested by the observations described. This may be facilitated by a rapid survey of the facts which are to be interpreted.

1. *Paramecium aurelia* can reproduce indefinitely without conjugation under favorable environmental conditions. The so-called life cycle is non-existent.
2. Minor periodic fluctuations (rhythms) occur in the rate of reproduction.
3. The rhythms are the obvious physiological expression of periodic internal phenomena.
4. The internal nuclear phenomena comprise the formation of a complete new nuclear apparatus of micronuclear origin. This process we term 'endomixis.'
5. The essential cytological difference between endomixis and conjugation is the absence of the third division which in conjugation forms the stationary and migratory micronuclei, and of necessity the non-formation of a syncaryon.

Now it is important to emphasize again that this race of *Paramecium aurelia* has existed (so far) for over seven years and through more than 4500 generations in a perfectly normal manner without conjugation, during which time it has undergone endomixis frequently, undoubtedly on the average once each month. Further, conjugation has been successfully consummated by animals from this culture and therefore both endomixis and conjugation are normal phenomena in the life of this same race.

It seems clear then that this culture offers strong *physiological* evidence against the interpretation of either of the first two so-called reducing micronuclear divisions as actually being a *chromosome reducing* division. Since the nuclear changes in endomixis and conjugation are fundamentally the same except for the absence of the third micronuclear division in the former, it is justifiable to regard this third micronuclear division which occurs in conjugation as the one directly preparatory, from the standpoint of chromosome reduction and sexual phenomena, for the imminent accession of foreign chromatin in the form of the migratory micronucleus of the other conjugant.

Endomixis, involving as it does the disintegration and absorption of the old macronuclear and micronuclear material, affords the opportunity for molecular rearrangement and therefore may afford the opportunity for the origin of variations within a pure

line of *Paramecium*. Jennings in his extensive studies on this organism has been able to discover only variations about the mean in pure lines without conjugation. These variations about the mean, we believe, are probably brought about, in part at least, by endomixis because, although there is no opportunity in this process for an introduction of foreign nuclear material, nevertheless there is an opportunity for its rearrangement. But Jennings admits that "There remains the possibility that heritable variations of a totally different (lesser) order of magnitude may arise during vegetative reproduction" ('13, p. 355). There is no evidence from the race of *Paramecium* which is the basis of our studies that variations in morphological features or in the division rate have occurred during its long cultivation which have been inherited—the animals of the 4500th generation being apparently in all ways similar to those of the early generations—but we wish to point out that, a priori, endomixis affords a field for the origin of such variations. It is conceivable that 'heritable' variations may result from some rare recombinations in endomixis. Biometrical studies of animals in isolated lines subsequent to endomixis are highly desirable.

In higher organisms fertilization has a dynamic as well as a hereditary rôle, and that these may be separated is amply attested by so-called artificial parthenogenesis. In the conjugation of *Paramecium*, Calkins, among recent investigators, has put emphasis on the dynamic aspect, though admitting the probability that conjugation is a source of variation; while Jennings definitely states that "There is no evidence that conjugation in the infusoria increases the reproductive power or rejuvenates the organism physiologically in any way" and puts all the emphasis on the side of variation and heredity.

Endomixis does initiate a new rhythm in the life history of *Paramecium*, i.e., a period of increased metabolic activity and therefore of reproductive activity, and since its fundamental morphological features are almost identical with those preliminary to the formation of the stationary and migratory micronuclei in conjugation, it lends strong support to the view that the dynamic aspect of conjugation is not absent.

From the life history of this race of *Paramecium aurelia* we lean toward the view that both endomixis and conjugation in the infusorian, as fertilization in higher forms, have a two-fold significance—both afford the opportunity for molecular rearrangement which leads to increased physiological activity, and the opportunity for variation. The life of the paramecium race can proceed indefinitely with endomixis under favorable environmental conditions—conjugation being unnecessary. “Senile degeneration” and “physiological death” are not the inevitable result of continued reproduction without conjugation—the cell has an internal regulatory phenomenon, endomixis, which is self-sufficient for the indefinite life of the race.

From whatever standpoint one views the wealth of data which has been secured during recent years in regard to the problems of variation, heredity, and the significance of conjugation in Protista, it is clear that hereafter, in the interpretation of previous and the prosecution of subsequent studies, due weight must be accorded to endomixis.

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EXPLANATION OF PLATES

All the figures represent specimens of *Paramecium aurelia*, except figure 34. The animals figured in red were fixed with sublimate-acetic and stained with Ranvier's picrocarmine; those figured in blue were fixed in Schaudinn's sublimate-alcohol and stained with Delafield's hematoxylin. The drawings were made from total preparations, with Abbe camera lucida, Zeiss homogeneous immersion 2 mm. and compensating ocular 12, with drawing board level with stage of microscope. Magnification, about 1500 diameters. Reduction for plates, one-half, which has obscured some micronuclear details.

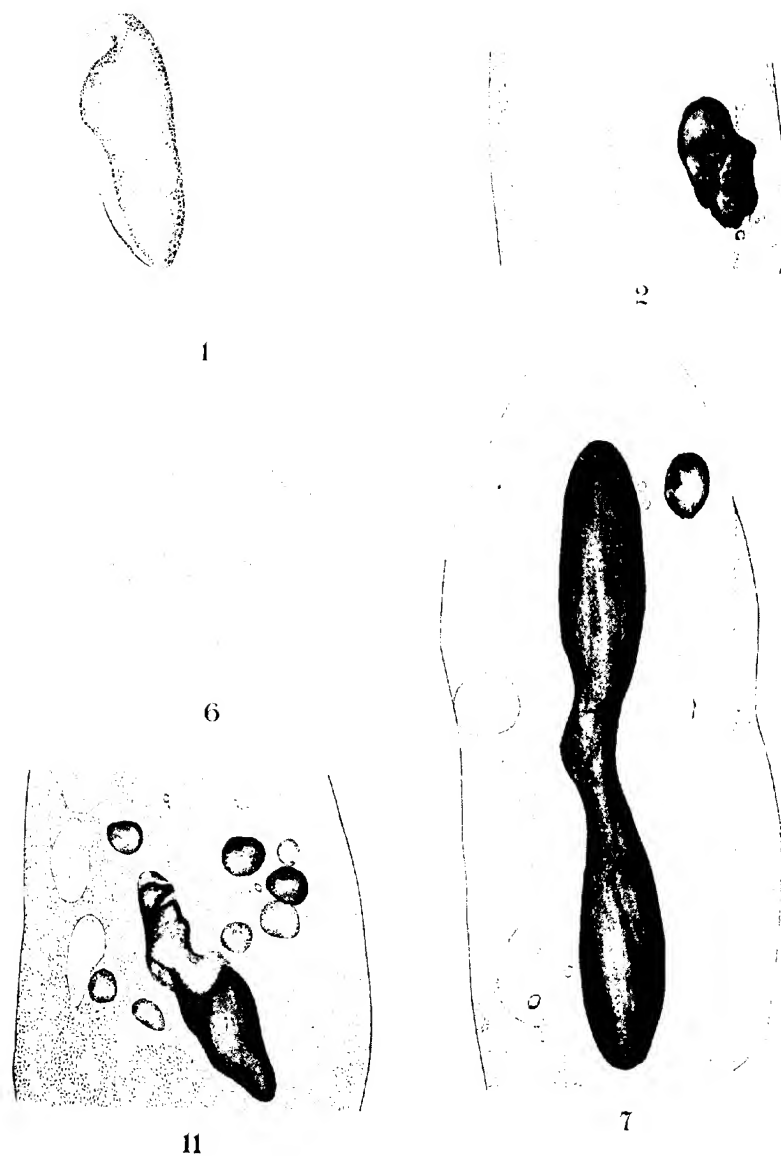
PLATE 1

EXPLANATION OF FIGURES

Descending Phase

- 1 Main Culture I, Line b, 424th generation, April 11, 1908. Normal animal in the period when the reorganization process is not in progress.
- 2 Subculture IE, Line VI, 4020th generation, October 27, 1913. Normal animal in the period when the reorganization process is not in progress.
- 3 Subculture IE, Line VIh, 4236th generation, February 21, 1914. Animal just before the beginning of the process in the 4238th generation. Macronucleus with coarse granules; two micronuclei which have shifted from their ordinary position close together at one side of the macronucleus.
- 4 Subculture IE, Line IV, 4044th generation, November 10, 1913. Small projections from the macronucleus; two resting micronuclei, and several vacuoles containing bacteria.
- 5 Subculture IE, Line VI, 4094th generation, December 9, 1913. Large projections from the macronucleus, which is coarsely granular. Two micronuclei shifted from their typical position.
- 6 Main culture I, Line c, 452d generation, April 30, 1908. Two chromatin bodies which have been ejected from the partially membranous macronucleus; two micronuclei shifted from their typical position.
- 7 Subculture IE, Line VI, 4182d generation, January 24, 1914. Animal in process of division during the descending phase of the process. One chromatin body, numerous vacuoles, four division micronuclei and the remnant of a division spindle.
- 8 Subculture IE, Line V, 4023d generation, October 30, 1913. Several chromatin bodies, some within and some which have passed out from the macronucleus. Two isolated micronuclei showing indications of division.
- 9 Subculture IE, Line II, 4069th generation, December 2, 1913. Macronucleus still further resolved into chromatin bodies; micronuclei are obscured by the macronucleus. Numerous vacuoles present.
- 10 Subculture IE, Line VI, 4130th generation, December 25, 1913. Numerous chromatin bodies the position of which in the cell indicates the effect of cytoplasmic currents. Macronucleus partially membranous. Animal started to effect the process which however was actually accomplished in this line in the 4140th generation. One micronucleus visible.
- 11 Subculture IE, Line IIIa, 4271st generation, March 12, 1914. Almost completely disintegrated macronucleus. Two micronuclei dividing to form four 'reduction' micronuclei; an irregular division in a third micronucleus.
- 12 Subculture IE, Line VIj, 4314th generation, April 4, 1914. Macronucleus nearly devoid of chromatin; numerous chromatin bodies; three micronuclei dividing to form six 'reduction' micronuclei.
- 13 Culture B, Berlin race, October 6, 1913 (see page 473). Numerous chromatin bodies; six so-called reduction micronuclei. Climax of process.

PERIODIC REORGANIZATION IN PARAMAECHUM
L. L. WOODRUFF AND RIL. ERDMANN



Woodruff, Erdmann and Bradley, del.

PLATE 2

EXPLANATION OF FIGURES

Climax

14 Main culture I, Line c, 426th generation, April 9, 1908. Old macronucleus merely in the form of a membrane from which the numerous chromatin bodies have been ejected and are free in the cytoplasm. Eight so-called reduction micronuclei two of which are lying isolated; the others in groups of three.

15 Main culture, Line b, 1498 to 1499th generation, December 12, 1909. A division stage at the climax of the process. Numerous chromatin bodies in each sister cell. One shows a membranous remnant of the old macronucleus. Each has only one micronucleus.

16 Mass culture started from main Culture I, October 12, 1913 (see page 435). One cell is in same stage as both cells in figure 15. The other cell shows the formation of the macronuclear anlagen, several chromatin bodies and two micronuclei.

17, 18 and 19 Culture B, Berlin race, October 6, 1913 (see page 473). Three animals illustrating the details of the formation of the macronuclear anlagen. Figure 17 shows two micronuclei, one macronuclear anlage completed and the other macronuclear anlage just beginning to be evolved from a micronucleus. Figure 18 shows two micronuclei and two completely formed macronuclear anlagen. Figure 19 shows the same except that the micronuclei have divided for the next cell division. In these three figures the chromatin bodies have been omitted. The cells have been counterstained with eosin.

20 Subculture IE, Line VIa, 4084th generation, December 4, 1913. Animal shows two macronuclear anlagen which have lost their initial form. Chromatin bodies beginning to disintegrate. Two micronuclei.

21 Subculture IE, Line VI, 4107th generation, December 16, 1913. The same stage as shown in figure 20, except that one of the micronuclei has completed division and the other is in process of division.

22 Subculture IE, Line III, 4063d generation, November 23, 1913. Essentially the same stage as former but showing both micronuclei in division.

23 Main culture I, Line c, 1755th generation, May 1, 1910. Animal figured to show that the complete reorganization of the cell occurred in the same manner at a relatively early period in the history of the main race.

24 Culture B, Berlin race, March 15, 1913. Animal figured to show that the complete reorganization occurred in an animal from a *different* race. Only one micronucleus is evident, the other being obscured by the chromatin bodies.

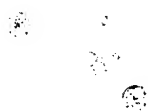
PERIODIC REORGANIZATION IN PARAMATHEUM
L. L. WOODRUFF AND R. L. ERMANN

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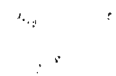


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PLATE 3

EXPLANATION OF FIGURES

Ascending Phase (Except figures 31 to 34)

25 Subculture IE, Line VIb, 4174th generation, January 18, 1914. Animal after the first cell division. One macronuclear anlage. Some chromatin bodies in process of disintegration and enclosed by vacuoles. One micronucleus has divided and the other is in process of division for the ensuing cell division.

26 Subculture IE, Line VI, 4185th generation, January 25, 1914. A characteristic stage of the ascending phase of the process. New macronucleus with fine chromatin granules. Two micronuclei present in cell but one near end omitted from drawing. Chromatin bodies disintegrating. Cf. sister cells, text fig. 8.

27 Subculture IE, Line IIIb, 4313th generation, April 3, 1914. New macronucleus, two micronuclei, four chromatin bodies and the cloud-like chromatin remnants of the others.

28 Main culture I, Line d, 1432 to 1433d generation, November 7, 1909. Young animals just after division during the ascending phase of the process. Micronuclei in each show various stages of division for the following cell division. The cell division just being completed is the fifth in about twenty-four hours (see page 456). A few chromatin bodies still present.

29 Subculture IE, Line VI, 4187th generation, February 27, 1914. New macronucleus, two micronuclei and three chromatin bodies.

30 Main culture I, Line b, 1201st generation, June 7, 1909. New macronucleus, two micronuclei and two remaining chromatin bodies one of which is under the macronucleus.

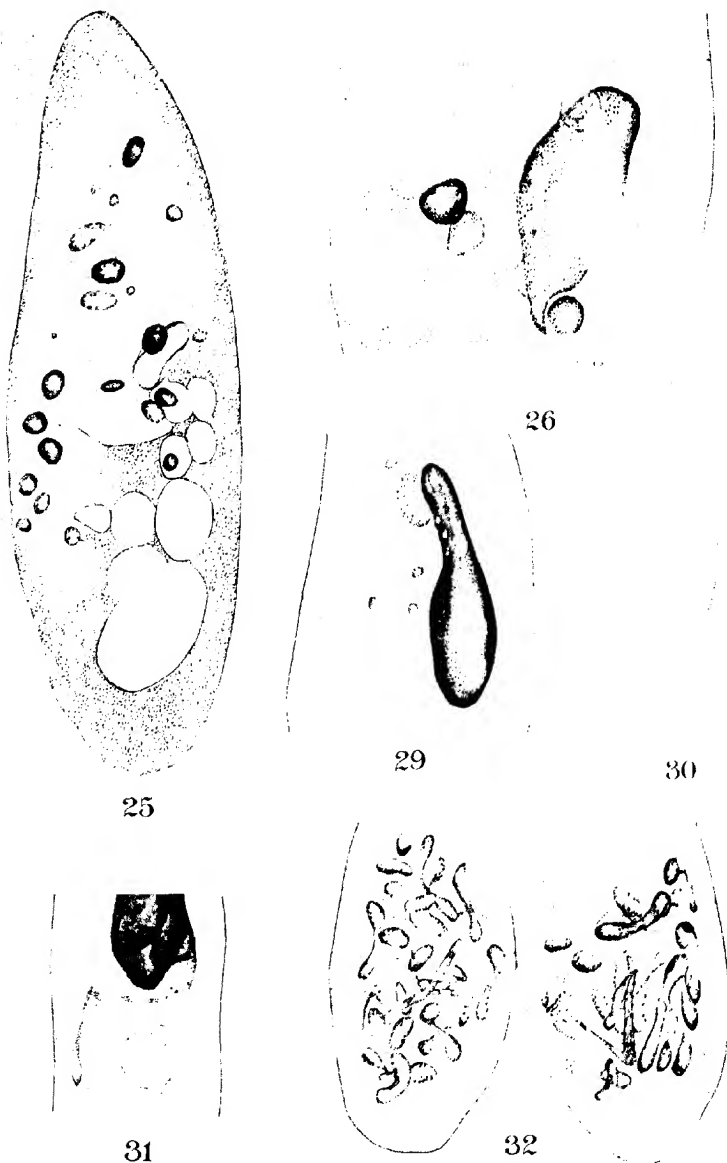
31 Mass culture seeded from main Culture I. An epidemic of conjugation in progress. Animal with micronuclei in process of division.

32 Pair of *conjugants*, showing first micronuclear division after formation of the syncyryon (cf. page 429 and page 453).

33 An *exconjugant* from isolated conjugating pair. First vegetative division completed. Macronuclear anlagen assuming typical macronuclear form. Note finely granular condition of chromatin typical of new macronucleus. Two micronuclei are present. Five food vacuoles with bacteria and promiscuous culture material in which conjugation was secured.

34 *Paramecium caudatum*, Culture Y, Line 2, 139th generation, April 29, 1914. Descending phase of the process showing the formation of the chromatin bodies in this species. The one characteristic micronucleus of *caudatum* has divided. Only optical section of micronuclei is drawn.

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PLATE 4

EXPLANATION OF FIGURES

Additional Stages

35 Subculture IE, Line VIb, 4092d generation, December 12, 1913. Typical form of macronuclear disintegration. Macronucleus small. Numerous chromatin bodies. Three micronuclei present.

36 Subculture IE, Line VI, 4102d generation, December 14, 1913. Typical form of macronuclear disintegration. Macronucleus of typical size, practically devoid of chromatin. Numerous chromatin bodies. Four reduction micronuclei; (cf. figs. 11 and 12). A₂, text figure 4, is sister cell.

37 Subculture IE, Line VIa, 4087th generation, December 6, 1913. An atypical form of macronuclear disintegration, slightly resembling the ribbon-like formation characteristic of conjugation. Beginning of micronuclear reduction.

38 Mass culture started from main Culture I, October 12, 1913. Transverse section through an animal at the climax. Seven chromatin bodies; two micronuclei in a cytoplasmic layer are visible. Stained with safranin.

39 Mass culture started from main Culture I, October 12, 1913. The posterior cell is in the same stage as both cells in figure 15. The anterior cell, after the formation of the macronuclear anlagen, has effected one more micronuclear division than the animal in figure 16a. The food vacuoles present contain remnants of material from hay infusion medium.

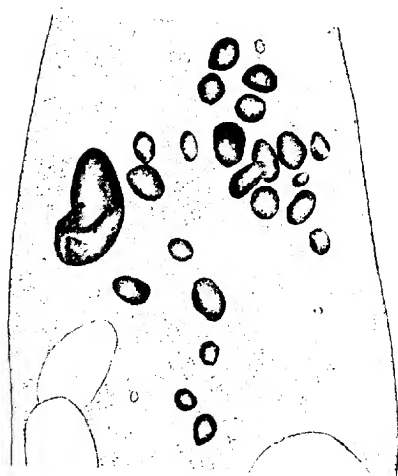
40 Subculture IE, Line VIh, 4355th generation, April 24, 1914. Stage at the end of the climax after the cell division characteristic of this period. Half the products of the next micronuclear division will become the two macronuclear anlagen.

41 and 42 Subculture IE, Line VIn, 4436th and 4437th generations, June 12, and 14, 1914. Cell 4436 has completed the cell division in the climax. Numerous chromatin bodies and one micronucleus. The sister cell in the 4436th generation, which kept the line alive, formed the macronuclear anlagen and by a division in about forty-eight hours distributed these to each of the two cells of the 4437th generation. One of these cells is shown in figure 42. Numerous chromatin bodies, one macronuclear anlage and four micronuclei. The micronuclear division indicates the quick succession of cell divisions characteristic of the ascending phase.

43 Subculture IE, Line VIq, 4437th generation, June 13, 1914. Animal after the first division in the ascending phase. New macronucleus with condensed chromatin. Three micronuclei.

44 Mass culture from main Culture I. Shows ribbon-like formation of macronucleus in a *conjugating* animal (cf. figs. 32 and 37). Eight reduction micronuclei, three of which are drawn.

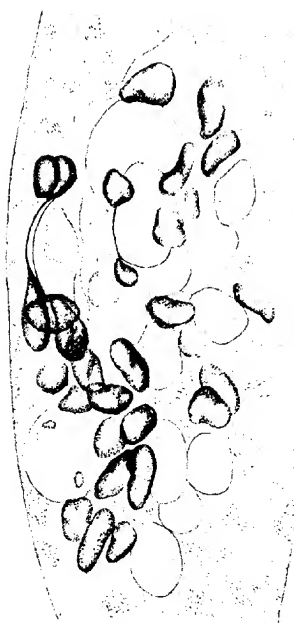
PERIODIC REORGANIZATION IN PARAMAECHTUM
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THE REACTION OF EMBRYONIC CELLS TO SOLID STRUCTURES

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FOURTEEN FIGURES

It is generally recognized that the movement of cells and cell masses is an essential factor in morphogenesis.¹ The most obvious movements concerned in the development of the Metazoa are those of cell aggregates, but these are often complicated by their association with growth or increase in mass. On the other hand, the movements of single cells, involving growth only to a very slight extent, are less complex and, at the same time, with our present methods of tissue culture, are readily amenable to observation and experiment. The mechanism of this movement is the streaming of the cell protoplasm, and the ontogenetic results depend upon the physical properties of the protoplasm itself and the stimuli which act upon it.

The importance of such factors in development was fully recognized twenty years ago by Roux² in his experimental study of the behavior of isolated cells of the frog's blastula, and by Herbst³ in his 'Reizphysiologie.' Even before this Loeb⁴ had considered the tropisms of cells, and had shown that the arrangement assumed by certain chromatophores in the fish embryo is dependent upon a stimulus emanating from the circulating blood. Somewhat later Driesch⁵ also took up the question and found a

¹ The various types of movement occurring in ontogeny have been carefully classified by Davenport in his "Preliminary catalogue of the processes concerned in ontogeny." *Bull. Mus. Comp. Zool. Harvard Coll.*, vol. 27, 1896.

² Cytotropismus. *Arch. f. Entw. Mech.*, Bd. 1, 1894; Bd. 3, 1896.

³ *Biol. Zentralbl.*, Bd. 14 and 15, 1894, 1895. Also *Formative Reize*, Leipzig, 1901.

⁴ *Jour. Morph.*, vol. 8, 1893.

⁵ *Arch. f. Entw. Mech.*, Bd. 3, 1896.

striking case of a cellular tropism (taktische Reizbarkeit) in the behavior of the mesenchyme cells of the sea urchin embryo. It must likewise not be forgotten that as early as 1892 Ramón y Cajal had stated his theory of chemotaxis to account for the outgrowth of the nerve fiber.⁶ Inexplicable as it may seem, very little of a definite nature has been added to our knowledge of this field since the period just cited.

In my work on the development of peripheral nerves⁷ it was definitely shown in confirmation of Cajal's theory, that the active factor in the spinning of the nerve fiber is a small mass of amoeboid protoplasm at the end of a cell process. The formation of long fibers by means of this mechanism was found to be a characteristic of embryonic nervous tissue, but other somewhat different forms of activity of the same general nature were seen in all cells of the frog embryo when cultivated in clotted lymph.⁸

No rigorous proof of the reaction of moving cells to definite stimuli was given in the course of these experiments. One of the most striking circumstances noted, however, was that when the lymph clotted firmly the movement was nearly always active, while in the cases in which the medium remained fluid only rounded cells were seen and these failed to undergo characteristic changes of form and locomotion. These observations led to the hypothesis that the cells were positively stereotropic and therefore unable to leave the solid masses to move out into purely fluid media. The work of Burrows with tissues of the chick embryo lent support to this hypothesis and it has since received further confirmation from other sources.⁹ Prior to this L. Loeb,¹⁰

⁶ La rétine des vertébrés. *La Cellule*, T. 9, 1892.

⁷ *Anat. Rec.*, vol. 1, 1907; *Jour. Exp. Zool.*, vol. 9, 1910.

⁸ Cells with processes of hyaline protoplasm were seen by Roux in the course of his work on cytotropism. The pseudopodia were described by him as occurring on cells that had become attached to the bottom. In one passage Roux states (*op. cit.*, p. 163) that it is doubtful if any cytotropic movements take place without the solid base.

⁹ Burrows, *Jour. Exp. Zool.*, vol. 10, 1911; Carrel and Burrows, *Jour. Exp. Med.*, vol. 14, 1911; Holmes, *Univ. California Pub., Zoölogy*, vol. 11, 1913.

¹⁰ *Arch. f. Entw. Mech.*, Bd. 6, 1897; Bd. 13, 1902; *Zeitschr. f. Krebsforschung*, Bd. 5, 1907.

from his studies on the growth of epithelium and carcinoma, was likewise led to the view that these tissues are stereotropic.

Nevertheless, none of these cases established the occurrence of this phenomenon beyond doubt. It seemed to be only a probable explanation of certain scattered facts. The experiments described in the present paper were designed to put the hypothesis to a more rigorous test.

Examples of stereotropism (thigmotaxis) have long been known in plants and there is a wide range of reactions in animals that may be gathered under this head, some of which have been considered by J. Loeb¹¹ and by Verworn.¹² These reactions, are however carried out mostly by complex mechanisms and have but little direct resemblance to the cellular phenomena here considered. The best known case of positive stereotropism in single cells is that of the spermatozoa of *Periplaneta*, originally described by Dewitz,¹³ who called attention to its significance in the act of fertilization. Jennings's¹⁴ description of the movements of amoeba indicate that here also reaction to solids plays an important part in locomotion.

METHOD OF EXPERIMENTATION

The plan of experimentation in the present work consisted in varying the three principal factors involved in the cultivation of tissues, viz., the tissue itself, the fluid medium, and the solid support. The results have shown that all three of these factors have some determining relation to the movement observed. Each tissue has certain characteristics, as seen especially in the differences manifested by epithelium, connective tissue and nervous tissue; the constitution of the fluid medium has a marked effect upon the vigor of the movement; the solid support influences the form and arrangement assumed by the moving cells, and its

¹¹ *Heliotropism der Tiere*. Würzburg, 1889; *Studies in general physiology*, Chicago, 1905; *Dynamics of living matter*, New York, 1906; Winterstein, *Vergleichende Physiologie*, Bd. 4, article on Tropisms.

¹² *Bewegung der lebendigen Substanz*, Jena 1892; *Allgemeine Physiologie*.

¹³ *Pflüger's Archiv.*, Bd. 38, 1886.

¹⁴ *Behavior of the lower organisms*. New York, 1906.

indispensability is shown by the fact that no movement takes place in its absence.

The first experiments were made with tissues from embryos of *Rana palustris* in the stage just after closure of the medullary folds. These were followed by a series of experiments with the tissues of chick embryos of various ages.¹⁵ The fluid media used were physiological sodium chloride, Locke's and Ringer's solutions in varying degrees of concentration, blood plasma and serum of the frog and the chicken respectively. The means used to support the planted tissues were the fibrin net work of the clotted plasma, spider web, the surface of the cover-glass, and, in some accidental cases, the surface film of the fluid drop. Control experiments were made in large drops of the fluid media, in which the tissue was prevented by the size of the drop from touching the cover-slip.

At the time the experiments with frog tissues were made it was not fully realized that the cover-slip might serve to support moving cells, so that in respect to the solid support only cultures upon spider web and in free hanging drops were made. In some of the latter the pieces of tissue came into contact with the cover-slip accidentally and cells began to wander out on the glass.

The technique used was in the main that employed in the study of the development of nerve fibers,¹⁶ together with the modifications introduced by Burrows.¹⁷ Sterilized apparatus and fluids were used throughout. The cultures were all made by the hanging drop method, the cover-glass being placed over the hollow of a deep depression slide, or upon a thin glass ring of about 20 mm. diameter and 2 mm. height and sealed on with vaseline. The frog tissues were kept at room temperature, while those

¹⁵ A preliminary account of the experiments with frog embryo cells was published in *Science*, vol. 34, 1911. The results upon chick tissues have been referred to briefly in several general papers (see *Anat. Rec.*, vol. 6, 1912; *Trans. Am. Cong. Phys. and Surg.*, 1913). The chick experiments were made with the assistance of Mr. (now Dr.) Paul G. Shipley, to whom it gives me much pleasure to express my thanks.

¹⁶ *Jour. Exp. Zool.*, vol. 9, 1910.

¹⁷ *Jour. Am. Med. Assoc.*, vol. 65, 1910; *Jour. Exp. Zool.*, vol. 10, 1911.

from the chick were incubated at about 39°C. The unusual hot weather which lasted during almost the whole period when the experiments with chick tissues were under way rendered unnecessary any precautions to keep the tissues warm during their preparation and examination.

The preparation of the spider web for the experiments was the only innovation in technique that needs special description. It was necessary to have the web tensely spread over a suitable frame so that it would support the drop of fluid from below. For this purpose glass rings were employed, since they were easy to sterilize and well adapted to form the wall of a moist chamber. A number of rings were placed in clean glass aquarium jars and a single spider introduced, which in the course of a day or two spun a web covering the whole bottom of the jar. The rings were then lifted out with forceps and the web cut or torn off around them. The slight roughness of the rim of the glass was sufficient to hold the web tense. The rings with the web upon them were sterilized by dry heat, the web standing a temperature of 150°C. without injury. In making the spider web preparations, the rings were first fastened to the slide with vaseline. A small drop of the culture fluid was then placed upon the web and the tissue afterwards introduced by means of a capillary pipette. It was unfortunately necessary to use very small drops of fluid since the weight of large drops caused the web to sag and touch the bottom of the chamber. The small size of the drops is sufficient to account for the relatively unfavorable results obtained by this method, the tissues usually not growing with very great luxuriance. After mounting the tissue upon the web, a cover-slip was placed over it and sealed on by vaseline. The weight of the cover-slip flattened out the small drop and the tissue was thus in contact both with the cover and the web. In most cases the covers themselves were coated with web so that the tissue was kept between two layers of this material. During the early part of the season there was some difficulty in finding a sufficient number of suitable spiders for the purpose.¹³

¹³ I am greatly indebted to Prof. A. Petrunkevitch for advice and assistance in collecting this material.

Tiginaria, which may be caught in old tree stumps, proved to be the best adapted of those which were obtainable early in the season, and two large specimens wove enough web for all of the experiments with frog tissues. Later the common grass spider, *Agalena*, was obtained in abundance. This form spins an extremely fine but dense web which is admirably adapted for the purpose. The individual threads of the web are amazingly thin and even under the oil immersion lens they appear as fine lines, the thickness of which can scarcely be measured.

EXPERIMENTS WITH TISSUES OF THE FROG EMBRYO

From this material seventy-one cultures were made in all. They are grouped in several series, of which the first two were of a preliminary nature, having been designed to ascertain what fluid media could be used.

Series I. Only inorganic media were used, as follows: tap water, 0.325 per cent NaCl, Ringer's solution without sugar, Locke's solution half diluted. Solid support was afforded in all cases by spider web. The tissues used were: medullary cord alone, medullary cord with axial mesoderm attached, and ectoderm. Eight preparations showed cell movements. Of the six that gave negative results, three were those which were mounted in tap water; they disintegrated on the second day. Two were in the dilute sodium chloride. One specimen in dilute Locke's solution lived seven days and the others from one to four days.

Series II. In these experiments both inorganic solutions (Ringer and 0.4 per cent NaCl) and defibrinated serum were used. Thirteen pieces were supported on web and eight were put up in hanging drops. None of the latter showed any movement of cells except one in which the drop touched the bottom of the chamber. In this some cell movement took place on the glass. Only one of the cultures in the saline solutions on the web gave positive indications of movement, the others showing no promise from the beginning. Of the nine in serum five were on web and four unsupported. Four of the former showed active movement, one was evidently injured since histolysis of the tissue began

almost immediately. None of the unsupported ones gave positive results.

Series III. This series of twenty-four experiments was designed to test rigorously the influence of the spider web upon the movements of the tissue, and as the preliminary experiments indicated that serum afforded a better medium than the inorganic solutions, the former alone was used. The serum was obtained from three different specimens of *Rana clamitans*, a species different from that of the embryonic tissue isolated. The cultures in this series were made in pairs. The central nervous system with some mesoderm attached was dissected entire out of the embryo in saline solution. It was then divided into two parts, one part being mounted upon web and the other in a plain hanging drop. In some cases the cephalic half of the medullary cord was placed on the web and the caudal half in the plain drop, while in others the order was reversed. The results of this series were entirely convincing. Out of the twelve preparations mounted upon the web eleven showed characteristic wandering of the cells with definite relations to the web fibers (fig. 1). The remaining one was disarranged accidentally the first day and gave no results. Cell movement began in some cases as early as eight hours after explantation and on the day following it was in full swing in all of the cultures. Of the twelve mounted in the large drops none showed any movement of cells except one of the cases in which the drop spread out, allowing the tissue to come into contact with the cover (fig. 3).¹⁹ In this one case six days after the culture was made a number of cells appeared on the cover and later some pigment cells were found. In the large drop preparations the isolated cells, which soon became very numerous,²⁰ always remained rounded (fig. 2), but in spite of considerable disintegration into single cells the main mass of tissue was in every case left intact. That it was alive was

¹⁹ The figure, which is much like the preparation referred to, was drawn from another case.

²⁰ In this respect embryonic frog tissues differ markedly from those of the chick, in which the elements remain closely bound together and the whole mass rounds off its outer surface (see p. 539 and figs. 11 and 14).

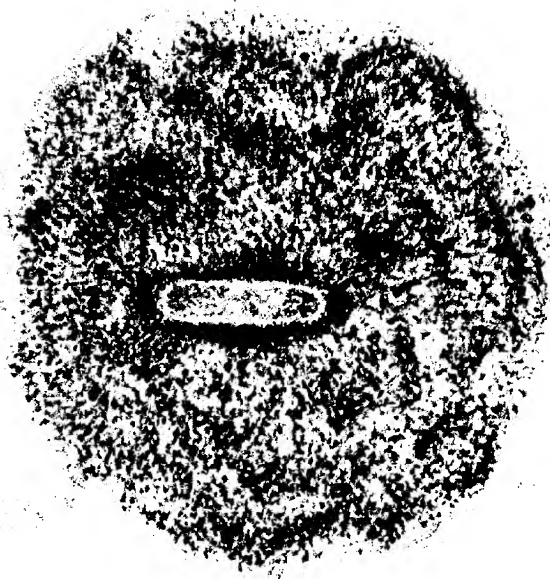
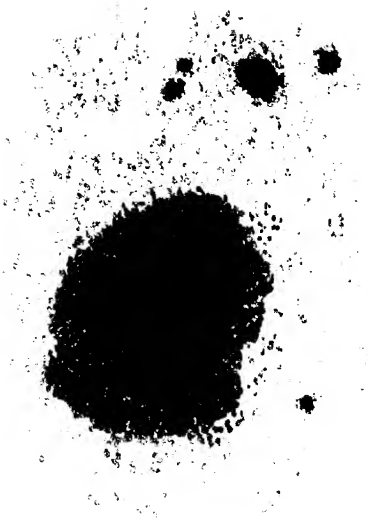


Unless otherwise stated figures were drawn from living specimens.

Fig. 1 Experiment S. W. 33. Medullary tube of embryo of *Rana palustris* cultivated 27 hours on spider web in serum from *R. Clamitans*. Some cells have moved out in sheets on the cover-slip; others are adapted to the web fibers. $\times 50$.

Fig. 2 Experiment S. W. 34. Medullary tube from same embryo, cultivated 28 hours in large hanging drop of same serum as in figure 1. Considerable disintegration of tissue, but isolated cells are rounded and exhibit no active movements. $\times 50$.

Fig. 3 Experiment S. W. 66. Medullary tube (and notochord) of *palustris* embryo cultivated two days in Locke's solution. The drop spread, leaving tissue in contact with cover. Detached cells are all on the glass. $\times 50$.



shown by the fact that the cells of the myotomes differentiated into muscle fibers, which after about five days began to contract sporadically, indicating a functionally intact neuro-muscular mechanism. The results of this set of experiments are summarized in table 1.

Series IV. The twelve preparations of this series were put up in the same way as the last except that Locke's solution was used instead of serum. The results were not so conclusive as in the last set, though they presented no contradictory evidence. Only two of the six spider web preparations showed cell movement in any considerable degree, two others were doubtful, while the remaining two gave no promise of activity at any time.

TABLE 1

Experiment III. Tissue in all cases medullary cord and myotomes

NO.	CHARACTER OF PREPARATION	CELL MOVEMENT TOOK PLACE	NERVE FIBERS FORMED	STRATIFIED MUSCLE FIBERS OBSERVED	MUSCLE TWITCHING TOOK PLACE	DURATION OF OB- SERVATION IN DAYS
31	Web	+				8
32	Round drop			+	+	22
33	Web	+				4
34	Round drop			+		22
35	Web	+				3
36	Spread drop					12
37	Web	+				2
38	Round drop				+	22
39	Web	+				29
40	Round drop				+	8
41	Web	+				6
42	Round drop				+	22
43	Web	+	+			6
44	Spread drop					12
45	Web	+	+		+	22
46	Spread drop					12
47	Web	+				12
48	Round drop				+	22
49	Web	+			+	20
50	Round drop			+	+	22
51	Web ¹					1
52	Spread drop ²	+			+	22
53	Web	+			+	22
54	Spread drop			+		22

¹ Injured.

² A few outwandering cells found on cover.

In the cultures mounted in plain drops the tissue was found in two cases to be in contact with the cover-slip, upon which a few cells wandered out from the main mass (fig. 3). A small number of nerve fibers were seen growing out upon the cover in one case. None of the others showed any cell movement. It is clear from the experiments that the inorganic media are not so favorable for the cultivation of tissues as plasma or serum.²¹

Table 2 gives a summary of the experiments with frog tissues.

The general character of the different types of culture is shown in the figures. Figures 1 and 2 represent two preparations from

TABLE 2
Summary of results of experiments with frog tissues

CHARACTER OF CULTURE	NUMBER OF PREPARATIONS	CELL WANDERING OCCURRED ON SOLID SUPPORT	NO CELL WANDERING	PERCENT POSITIVE ON SOLID SUPPORT
<i>Defibrinated serum</i>				
Spider web.....	17	15	2	88
Large drop.....	11		11	
Spread drop.....	5	1	4	20
<i>Saline solutions</i>				
Spider web.....	25 ¹	11	14	44
Large drop.....	5		4 ²	
Spread drop.....	5	2	3	40

¹ Exclusive of three cases in tap water in which disintegration began very early.

² In one case tissue touched bottom of chamber and some outwandering occurred.

Series III mounted respectively upon spider web and in a plain drop. In the latter (fig. 2) there are a great many loose cells which have separated from the main mass of tissue and assumed spherical shape, while the main mass itself looks to be upon the point of disintegration, though, as subsequent observation showed, this did not take place. The drawing was made about twenty-eight hours after the tissue was prepared. In figure 1 the companion preparation is represented. The mass of tissue is held against the cover-slip by the spider web and is much more flat-

²¹ Cf. M. R. and W. H. Lewis, *Anat. Rec.*, vol. 5, 1911.

tened than in the other case. A comparatively thin fringe of cells has formed around it and many cells have left the mass entirely, being scattered at various distances from the latter. These cells are at two levels. The upper layer is in contact with the lower surface of the cover-slip, where the outwandering cells are apt to assume a polygonal form, though they are sometimes influenced by the web fibers attached to the cover. In the lower layer the cells are arranged with reference to the web fibers, and they are usually drawn out into long processes which are closely applied to the latter (see also figs. 4 to 7).

The preparation shown in figure 3 is from Series IV, being one of those mounted in Locke's solution. It shows the effect of contact with the smooth cover-slip only. The drawing was made about forty-five hours after the culture was prepared. The tissue consisted of a piece of the medullary cord with some mesoderm and a small piece of the notochord attached. It has been flattened in pancake form against the cover-slip by the spreading of the drop of fluid. A small number of isolated polygonal cells have moved off upon the cover, and a thin fringe of cells surrounds the tissue as in the preceding case.

The adaptation of the cells to the web fibers is shown more clearly in figures 4 to 7, which are all taken from the same series of experiments. Figure 4 shows a cell with two hyaline processes attached to the web fibers running approximately at right angles. Figure 5 shows two bipolar and a tripolar cell, drawn when the preparation was two days old, figure 6 two cells from an eight day culture, and figure 7 pigment cells from another preparation of six days. The pigment is of two kinds, a granular black melanin and a diffuse yellow pigment (probably a lipochrome) present in two of the cells in large quantities. The yellow cells contain a little of the dark granular pigment, though much less than the other cells, and the latter contain none of the yellow.

Nerve fibers developed in only a few of the cases and in none of these was anything notable shown. All of the nerves observed were growing upon the surface of the cover slip. The web fibers do not seem to be a favorable support for them.

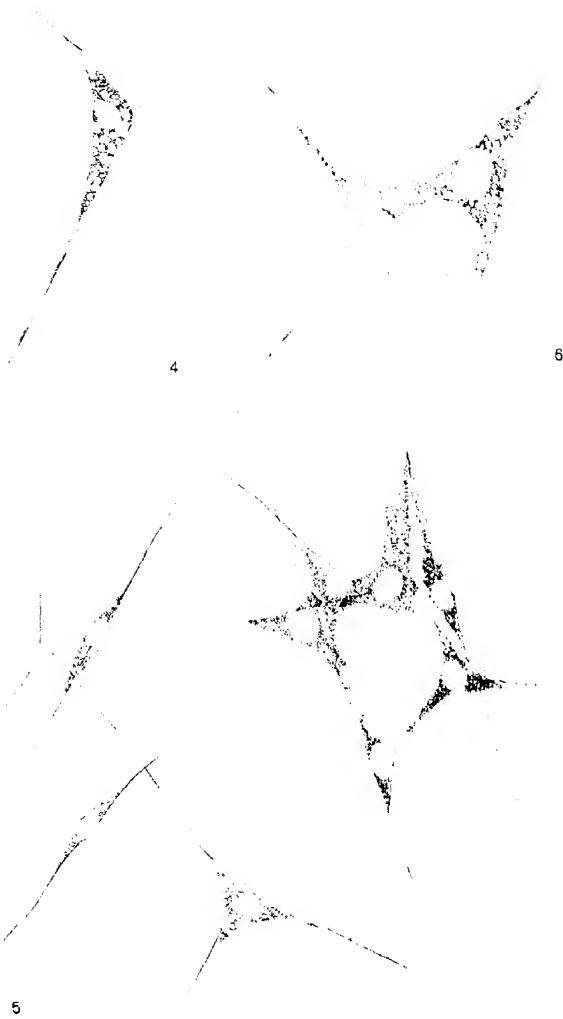


Fig. 4 Experiment S. W. 47. Cell from medullary cord with two processes attached to crossed web fibers. $\times 300$.

Fig. 5 Preparation S. W. 53. Bipolar and tripolar cells from medullary cord attached to spider web fibers two days after explantation. $\times 300$.

Fig. 6 Experiment S. W. 39. Similar preparation eight days old. $\times 300$.

Fig. 7 Experiment S. W. 49, showing the two types of pigment cells; six days old. $\times 300$.

EXPERIMENTS WITH CHICK TISSUES

One hundred and two experiments divided into ten series were made with chick tissue, comprising altogether 142 different cultures since in some cases more than one culture was made under the same cover. In three of the series the medium used was Locke's solution, and in the other seven it was defibrinated serum. Clotted plasma was used for comparison in all series. When plasma and serum were used the blood was always taken from the same hen so as to have an identical fluid medium in all cases.

Fixed support for the tissues was afforded by spider web prepared as described above, by clotted plasma and by the lower surface of the cover-slip. The latter was rendered available by mounting the tissue in small drops. In other cases contact was brought about after a time by the spreading of the drop which was originally large. This gives a firm attachment but rarely a luxuriant growth because of the extreme thinness of the fluid film.²² Tissues from embryos varying in age from two to nine days were used. The results are given briefly in table 3, which shows the behavior of cells in the different media without, however, attempting to analyze the behavior of different tissues. Plasma preparations are 100 per cent positive. The cover-slip preparations (i.e., those mounted in small drops) show also a very high percentage of positive results while the spider web cultures are less favorable, due probably as pointed out above, to the extremely small amount of fluid used. The large drop preparations are all negative except two which show some cell wandering on the surface film. Several of the small drop cultures also show slight cell movement on the surface. The film cannot, however, be a very favorable surface for movement; otherwise we should expect to find cells upon it much more fre-

²² Burrows (Trans. Am. Cong. Phys. and Surg., 1913) has shown that the amount of cell migration depends upon the thickness of the layer of medium. There is an optimum thickness above and below which less movement takes place; in the thick layer because of insufficient oxygen and lesser concentration of repelling waste products (acid), and in the very thin layer (below the optimum) because of the small amount of nutrient medium.

TABLE 3

Summary of results of experiments with chick tissues

CHARACTER OF CULTURE	NUMBER OF PREPARATIONS	CELL WENT DURING ON SOLID SUPPORT	CELLS ON SURFACE FILM	SAFETY WANDERING	PERCENT POSITIVE ON SOLID SUPPORT
Plasma clot.....	34	34			100
<i>Defibrinated serum</i>					
Spider web.....	31	23		8	74
Large drop.....	14		1	13	
Small drop.....	5	5			100
Spread drop.....	6	1		5	17
<i>Locke's solution</i>					
Spider web.....	30	19		11	63
Large drop.....	7		1	6	
Small drop.....	12	10	2	1	83
Spread drop.....	3	3			100

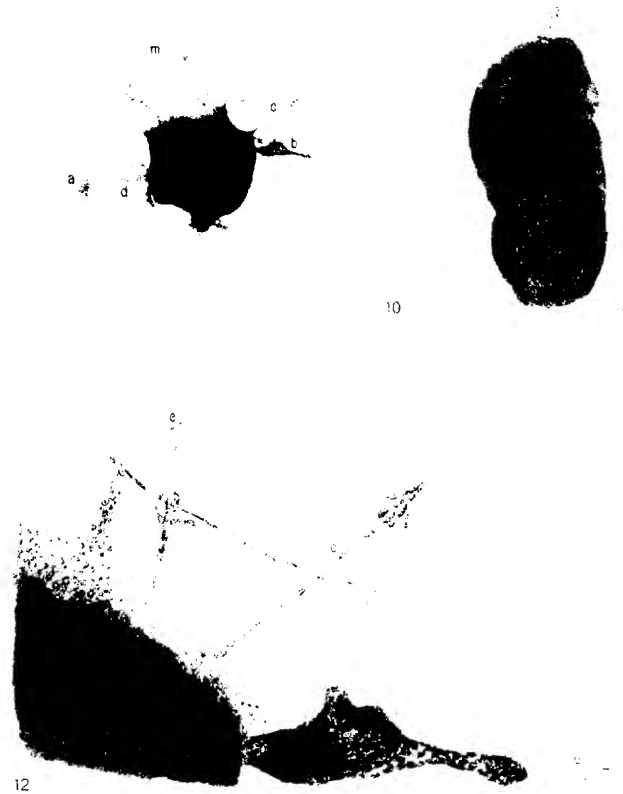
quently. It is of interest to note that in several of the large drop preparations where the tissue was at first not in contact with the cover, it afterwards did touch the glass and then cell movement began. These have been classed with the small drop (contact) group.

The main results of these experiments can best be presented by the description of four different cases taken from the same series and shown in figures 8 to 11. The cultures are all from pieces of the duodenum of a nine-day chick embryo, and the drawings were made from specimens preserved two days after preparation.

The first (fig. 8) has been cultivated in clotted plasma. In this there is a very characteristic ring formation with bands of tissue extending across the clear space. Isolated mesenchyme cells are present and the epithelium (endothelium?) shows its usual tendency to form membranes.

The second (fig. 9) was cultivated in a small drop of defibrinated serum and the tissue was in contact with the coverslip from the beginning. The striking feature of this case is the formation of a wide membrane extending out from nearly the whole circumference of the original tissue. In one region conditions have prevented this movement, but at a distance from





Figs. 8 to 11²³ Duodenum of nine-day chick embryo cultivated with different kinds of solid support. Drawn from specimens preserved two days after explantation. $\times 39$.

Fig. 8 In clotted plasma.

Fig. 9 In serum in contact with cover glass.

Fig. 10 In serum on spider web.

Fig. 11 Free in large hanging drop of serum.

Fig. 12²³ Portion of figure 10 under high power. $\times 300$. Explanation of details in text.

²³ These preparations, which were used to illustrate a general paper read before the American Congress of Physicians and Surgeons, have been redrawn (except fig. 31) for the present account in order to show greater exactness of detail.

the main mass the membrane from the two adjacent sides has united, leaving a large hole, and there are also several smaller spaces left uncovered. A considerable number of isolated branched mesenchyme cells are to be found outside the area covered by the membrane. All of these structures are fixed to the cover-slip. The cells forming the membrane are one layer thick.²⁴ Those near the periphery of the membrane have the greatest superficial area, and there is a general decrease in their dimensions as the tissue mass is approached, though there are irregularities in this respect. There are no mitoses present, which indicates that membrane formation is due to movement of cells and not to growth.²⁵ There is no way of ascertaining whether this particular membrane is endothelial or from the epithelium lining the intestinal tract.

The third culture (fig. 10) was supported upon spider web in the manner described above. It does not show such extensive cell wandering as some other preparations but it is very characteristic. A membrane (*m*) has been formed on the cover glass at one side of the preparation, and at another place, quite detached from the main mass, there is a small group of cells (*a*) which have moved out along the web fibers. On the opposite side of the tissue a considerable mass of cells (*b*) projects sharply outward and is firmly attached to the web. What is most interesting in this case is the banding together of spindle cells upon bundles of web fibers or upon single threads to form structures which closely simulate embryonic nerves with their sheath cells (*c*). In several places web fibers covered with such cells cross one another and here there is some accumulation of cells. Spindle shaped cells are also found singly (*e*). Some necrotic tissue (*d*) is present at places around the periphery of the main mass. The arrangement of these structures is shown in figure 12, which represents part of the preparation under higher magnification.

²⁴ Cf. M. R. and W. H. Lewis, *Anat. Rec.*, vol. 6, 1912. Also Burrows, 17th International Congress of Medicine, London, 1913; Section General Path. and Path. Anat., p. 225.

²⁵ This is in harmony with the observations of many authors on the covering of wound surfaces by epithelium.

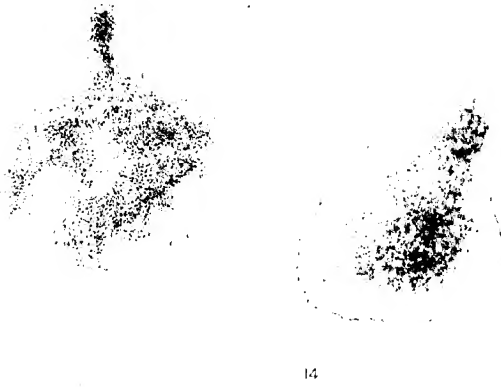


Fig. 13 Experiment C 19. Piece of telencephalon of five-day chick embryo cultivated two days in clotted plasma, showing fringe of nerve fibers growing out into clot. $\times 39$.

Fig. 14 Experiment C 15. Piece of telencephalon of five-day chick embryo cultivated two days in defibrinated serum. $\times 39$.

In the fourth case (fig. 11), which was cultivated in a large drop where the tissue never was in contact with the cover glass, there are no evidences of cell wandering whatever. The tissue is rounded off and has an almost smooth contour.

One series of experiments was made to test the effect of the different kinds of support upon the growth of nerve fibers. Pieces of the telencephalon of a five-day embryo were used and twelve cultures were made, four of each kind, in a large drop, on spider web and in clotted plasma respectively. Those mounted in the plasma clot all showed the development of nerve fibers one day after explantation. In the more favorable cases they formed a veritable fringe around the whole periphery of the specimen. The fibers were much matted together and some were branched (fig. 13). Neither the preparations on web nor those

in the large drop showed the same condition. Two of the former showed cell movement, i.e., some wandering upon the cover and the web fibers but no nerves were present. In all of the large drop preparations (fig. 14) the contour of the tissue was relatively smooth and no cells wandered out. These experiments tend to corroborate the results obtained with frog tissues, that the web fibers are not favorable to the outgrowth of nerves, though they are too few in number to be at all conclusive in this respect.

DISCUSSION

The foregoing experiments lead to the conclusion that solid objects are an important and even necessary factor in the movement of embryonic cells, such as mesenchyme and epithelium. Leaving out the cases of movement upon the surface film, which, moreover, has certain properties of solids, there are no exceptions to the rule that movement takes place only when contact with solid material is attained. Furthermore, each of the three kinds of solid support used in the experiments influences the cell movement in its own way, as is shown by the different arrangement assumed by the cells after a certain period of cultivation (figs. 8 to 10). The reactions to solids take place whether the fluid medium is a saline solution or serum, though the latter is conducive to more active movement (and growth) than the former, owing no doubt to its nutritive qualities.²⁶

The question now arises whether these reactions are to be regarded as a manifestation of stereotropism (thigmotaxis), which is a response to mechanical stimulation (pressure), or whether the solid acts only indirectly by inducing conditions that give rise to chemical or some other form of stimulation. Burrows²⁷ has shown that the centrifugal movement of cells observed in almost all cultures, i.e., the movement from the implanted cell mass out into the culture medium, may be explained by the acidity produced in the main mass of cells through the accumulation of waste products. That a condition of acidity does

²⁶ Cf. M. R. and W. H. Lewis, *Anat. Rec.*, vol. 6, 1912.

²⁷ *Proc. Am. Cong. Phys. and Surg.*, 1913.

obtain in the center of tissue cultures after a few hours of incubation has been proved by means of indicators.²⁸ A given cell would thus be exposed on one side to a more acid medium diffusing from the tissue, and on the other to the more alkaline culture medium. This condition would, in accordance with the Quincke-Bütschli surface tension theory of amoeboid activity, bring about the formation of pseudopodia on the side of the cell turned toward the fresh medium and thus produce a centrifugal movement. Similarly the flattening of an isolated cell or the spreading of a sheet of cells on a smooth surface would be accounted for. Burrows thinks that no movement takes place as the result of mere contact with solids without the secondary stimulus resulting from the chemical change.

These considerations at least show that even under very simple conditions, where no chemical stimuli have been intentionally applied, the local activities within a culture may nevertheless give rise to such conditions as might stimulate the cells chemically on one side and thus direct cell movement. It however remains a fact that the chemical stimuli in question are powerless to call forth these movements in the absence of solid support, else the large drop cultures would not behave as they do. The acidity theory also offers no adequate explanation of the adaptation of single cells to such minute structures as the web fibers, nor of the fact that outwandering cells rapidly bridge a gap between two separate pieces of tissue in the same culture. While it must therefore be admitted that chemical stimuli may play an important part in influencing the movements of cells in simple cultures, as Burrows has pointed out, the facts show that the cells are also stimulated by solids as such and respond to them by an orienting movement.

Response to tactile stimuli is of such general occurrence in animals that there is nothing anomalous in the manifestation of the same kind of sensitiveness in cells. Though in the metazoa the responses are brought about by complex neuro-muscular mechanisms, the reactions to mechanical stimuli given by tissue cells and the protozoa are closely comparable with one another.

²⁸ Cf. Burrows, *ibid.*, and Rous, *Jour. Exp. Med.*, vol. 18, 1913.

The latter have been studied by Verworn²⁹ in Rhizopods and by Pütter³⁰ in Flagellates and Ciliates. These observers have shown that different parts of the same organism respond differently to contact stimuli. In Protozoa, where the movement is sufficiently rapid to be directly observed, the location within the cell of both the sensitive region and the responding mechanism can often be made out, whereas it has as yet been found impossible to do so in the case of the slowly moving tissue cells. This is a difficulty that applies alike to the study of the reactions to all kinds of stimuli and renders uncertain for the present any attempt at exact description of the process. At the same time the case of *Chilomonas*, for instance, in which according to Pütter stimulation of one of the flagella by solids calls forth a positive response, while similar stimulation of the other a negative one, shows that we have in this kind of sensitiveness an important directive factor. By analogy in the case of tissue cells, if different parts of the same cell are unequally sensitive, it is likely that contact with an even surface would induce movement in a definite direction. Barring some such regional difference in the irritability of the cell, the kind of reaction called forth by contact with a solid object could only be a clinging to that object or a recoil from it. The former or positive reaction is that shown by the embryonic cells here studied. Without any further stimulus it is conceivable that the reaction to such contact might result in the change of shape of the cell, either its flattening upon the surface of a glass cover-slip or its elongation upon the fibers of the spider web (fig. 12). Its locomotion, however, must depend either upon some other stimulus or upon some local internal differences in the cell.

As to the propriety of calling the reaction to solids a tropism, the position here taken differs from that of Loeb who holds that it is no real tropism, in as much as lines of force do not exist.³¹ It is true that in the case of tactile stimuli the source of the

²⁹ *Allgemeine Physiologie*, 5th Edition, p. 519; *Bewegung der lebendigen Substanz*, Jena, 1892.

³⁰ *Arch. f. Anat. u. Physiol. physiol. Abt. Supplementband*, 1900.

³¹ *Dynamics of living matter*, New York, p. 156.

stimulus is not placed at a distance from the organism, for it is the relative motion of the organism stimulated and the stimulating object acting only when they come into contact, that gives rise to the stimulus, and in the case of a moving organism coming into contact with a fixed object the energy is supplied by the organism itself. However this is not sufficient ground for drawing general distinction between the response to such stimuli and the reaction to a beam of light. The difference lies only in the source of the stimulus itself, i.e., in the distance from which it comes, and in the continuousness of its effect. In the case of a negative reaction to pressure the stimulus must cease as soon as the recoil is made. In case of a positive reaction the stimulus may continue but there can be no further visible effect beyond the change in shape of the organism unless its parts are differentially sensitive. The similarity to phototropism becomes closer in the case of rheotropism or reaction to currents in the surrounding medium, which, as Verworn pointed out,³² is to be classed under the group of responses to mechanical stimuli.

A word remains to be said regarding the significance of the facts brought out in this study with reference to the interpretation of some of the phenomena of normal development. The resemblance between the arrangement of cells on the web fibers (especially as seen in figure 12) and the sheath cells of an embryonic nerve suggests that stereotropism may have something to do with the latter, though not necessarily to the exclusion of chemotropic influences. Similarly the close application of mesenchyme cells to such structures as blood vessels, muscles, and various other organs, resulting in the formation of a cellular sheath, which afterwards becomes skleratized, may be due in the first instance to a stereotropic response. In fact the conditions within the embryo at the time when the tissues are taken for the experiments are such as to suggest that this stereotropic reaction is an important factor in the behavior of many kinds of cells. There is a very active movement of mesenchyme at the time, during which cells stream from certain regions and fill in the

³² Allgemeine Physiologie.

spaces between the main organ systems of the body which are already laid down. In this process the surface of structures such as the medullary cord, notochord, alimentary canal, muscle plates and the inner surface of the epidermis would serve as a solid base upon which the cells might creep. It is found that sooner or later all of these surfaces become ensheathed or lined by connective tissue cells. In the encystment of foreign bodies within the organism a similar phenomenon is observed.

With regard to the movements of the growing nerve fiber the evidence, as pointed out above, is not quite so varied, but it is sufficient to warrant the conclusion that also this protoplasm is stereotropic. No free outgrowth of nerves in a fluid medium has ever been observed, while such solids as the fibrin clot and smooth glass surfaces serve readily to support them, as do the surfaces of the larger cell masses and the interstitial protoplasmic network inside the embryo.

Most of these points were discussed some years ago by Herbst,³³ who, however, did not claim to have reached a definite conclusion as to the exact kind of reaction involved. The experiments here described do not of course settle the question either, but since it has been shown that most embryonic cells are stereotropic, and that such arrangements as they assume in the embryo may often be induced under cultural conditions by reactions to solids, there is a presumption in favor of the view that this type of reaction is a potent factor in normal development also. Inferences as to what goes on in the embryo which are not based upon exact information regarding the physiological properties of the tissue elements are likely to prove erroneous. On the other hand, if we know the actual properties of individual cells in detail, it will be possible to form, on the basis of the observation of normal development, an accurate conception of the influences actually at work in shaping the embryonic body.

³³ Biol. Centralbl., Bd. 14, pp. 746.

THE INFLUENCE OF FOOD IN CONTROLLING SEX IN HYDATINA SENTA

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Considerable interest has been manifested concerning the sex ratio in the parthenogenetically produced male and female individuals in the rotifer *Hydatina senta*. At one time in general cultures of rotifers only females are found, at another time females and males are found in equal numbers, and at still other times very few females but from 80 to 90 per cent of males are found.

The problem has been to determine the causes that regulate the production of the two sexes in this rotifer—why at one time there is produced an excess of females and at another time an excess of males.

The results of the work of earlier investigators on this problem having been reviewed so thoroughly and frequently recently, only references to them in the bibliography will be made at this time.

Whitney made an extensive series of experiments and observations in regard to temperature and starvation and came to the conclusion that neither factor was influential in the regulation of the production of the two sexes. He, moreover, found that the so-called female sex strains of Punnett could be made to produce many males by changing the environment. However, he was unable to discover the real factor that changed the female sex strain into one that produced many males, but he was of the opinion that whatever the potent factor was that sometimes caused only females to be produced and at other times caused nearly all males to be produced, it must be an external factor. Moreover, he made observations on one strain through 289 generations, for about two years, in which no males were

produced. This was accomplished by using a continuous and uniform diet of the flagellate, *Polytoma*, reared in a solution of cooked horse manure. Throughout this time in side experiments on this strain males were produced in large numbers many times by transferring the rotifers to a new mixed food culture.

Shull carried on many experiments with temperature, starvation, and various chemicals and finally corroborated the general conclusions of Punnett and Whitney that temperature and starvation were negative factors in the regulation of the two sexes. He, however, was able to demonstrate that the unknown factor must act upon the grandmother and not upon the mother in order to cause abrupt changes in the sex ratio among the progeny. In this point he was in agreement with Maupas' work. However, Shull finally concluded that this influential factor that controlled the production of the two sexes was an *internal* one.

Mitchell in working with the rotifer, *Asplanchna*, recently has found that a continuous diet of a uniform food culture will cause only females to be produced, but if the diet is changed to a new food, males are produced in the third generation (grandchildren). He suggested that a change of food might be found to be the controlling factor in regulating the production of the two sexes in *Hydatina senta*.

In the experiments of the previous workers upon *Hydatina senta* very little attention has been given to any particular kind of food. Usually any mixed Protozoa culture upon which the rotifers would thrive was used. However, Shull and Whitney used a more or less pure culture of the colorless flagellate, *Polytoma*, but obtained varying results. This may be due to the fact that these cultures in some instances were mixed cultures to a considerable extent although not suspected to be mixed. Whitney's cultures were probably the same throughout the two-year period in which he obtained 289 generations of females. Uniform conditions produced uniform results. At that time this point was not fully appreciated.

About five years ago the author had a general culture of rotifers in a jar which was placed in a south window in the laboratory and remained there during the spring months. Some time in May during a period of a few days the jar was swarming with countless numbers of the green flagellate, *Dunaliella*, Teodor (*Chlamydomonas*, Cohn). On one day thirty-two female eggs of the rotifers were taken from the surface of the culture water and placed in watch-glasses and allowed to hatch. Each young female matured and thirty (93+per cent) of these females produced male offspring. This *Dunaliella* culture soon disappeared and no other good one ever appeared again. However, the author saved a little of the culture and spent the entire summer in the attempt to grow pure or even mixed cultures of it. No medium was found in which it would grow.

This year another attempt has been made to grow cultures of *Dunaliella* and has been quite successful although the method is not yet completely perfected. They were raised in countless numbers in direct sunlight and a solution of bouillon. An Armour's bouillon cube was dissolved and boiled in 400 cc. of tap water and equal parts of this bouillon solution and of sterilized water were used for the culture medium. The medium was inoculated with a few individuals of *Dunaliella* and the jar placed in a south window. Within 7 to 14 days the surface of the culture water was swarming with the active *Dunaliella* during the sunny part of the day. Toward night the animals became inactive and motionless, in which state they remained until the following morning and then only became thoroughly active again provided there was direct sunlight. After the culture became 2 to 3 weeks of age the *Dunaliella* would remain continually in the motionless state. At this time the old culture water was siphoned off and some new culture medium was added to the quiescent *Dunaliella*. Within 2 to 3 days, if in sunlight, practically all the *Dunaliella* would be active. The surface was removed with a pipette and placed in a test-tube and by centrifuging, all the *Dunaliella* were collected at the bottom of the test-tube. Then nearly all of the liquid in the test-tube was poured off, thus leaving the *Dunaliella* in enormous

numbers in a small amount of the culture water. Three or four drops of this material was added to about 8 cc. of old filtered culture water in which a general mixed culture of Protozoa and rotifers were living and placed in watch-glasses. These glasses were placed in sunlight at a temperature of about 25°C. and the *Dunaliella* remained active as long as they were in the sunlight.

It, however, usually happened that when these watch-glasses were left in the sunlight for any appreciable length of time, the temperature would rise to 31 to 40°C., which would be fatal to the rotifers. To obviate this difficulty, a smaller battery jar was set and fastened into a larger battery jar which had padding in the bottom of it. This allowed the top of the smaller jar to protrude above the top of the larger jar. A space was thus made between the two jars into which running water was conducted, and by regulating the flow the temperature could be kept at almost any point desired while this water-jacket jar was in direct sunlight. The rotifers in the *Dunaliella* culture were placed in the inner jar in watch-glasses and the jar was covered with a glass plate. By this method a constant temperature of about 25 to 26°C. was maintained, at which degrees the *Dunaliella* were the most active in swimming about in the culture water. The rotifers eat them only when they are active, chiefly because when they are motionless they form a scum at the surface of the culture water or become fastened to the sides of the dish and in either place they are inaccessible to the rotifers.

At the beginning of the experiments a female rotifer was taken from a jar containing a general culture of rotifers which had developed from fertilized eggs a few months previously. This female was fed upon pure cultures of the colorless flagellate, *Polytoma*, and became the progenitor of a parthenogenetic strain of rotifers which produced almost entirely female offspring through twenty-five generations. The method of causing a strain to produce only female offspring has been described in a former paper, but as the method of making the food cultures has been improved it will be briefly described again. Pure cultures of *Polytoma* were made in the following manner: 800 grams of fresh horse manure were put into 1200 cc. of water and

cooked in a steam sterilizer at 10 to 15 pounds pressure for one hour. The liquid part was then pressed out and usually equalled 1000 cc. This was put into a flask with a cotton plug and sterilized. This could be kept indefinitely and used as a stock supply provided it was sterilized each time after it was opened. One part (100 cc.) of this liquid was added to three parts (300 cc.) of sterilized water, inoculated with a few *Polytoma* and placed in a large flat dish, thus giving a large surface of the culture water exposed to the air. Within 2 to 3 days the culture water would be teeming with the *Polytoma* but after a day or two the culture became spent and only a few *Polytoma* would be found. When this culture was about three days old one-half of it was poured off and 100 cc. of the stock supply of the manure liquid and 300 cc. of sterilized water were added. Eighteen to twenty-four hours later countless numbers of the *Polytoma* could be taken off from the surface with a pipette. This process of pouring off one-half of the old culture water and adding new medium was repeated every day and in this way a vigorous food culture of *Polytoma* was continuously maintained for the rotifers at room temperature.

Female rotifers of various ages were taken from the controlled female strain, which produced nearly all females through twenty-five generations by being fed upon a continuous diet of *Polytoma*, and fed the green flagellate, *Dunaliella*, by the method as described earlier in this paper.

Tables 1 to 5 show the important results obtained in a long series of observations.

Experiments 1 to 23 have been omitted because they were made when the methods of feeding were being perfected and consequently did not show as favorable results as the later experiments. Experiments 24 to 63 include all the other experiments which were made in a successive series. A large number of individuals, considering the amount of work that each one entailed, has been observed, as recorded in tables 1 to 3. 341 mothers produced 5562 daughters, of which 3 per cent were male-producing daughters when reared under the influence of *Polytoma* diet, and of which 57% were male-producing daughters

when the diet of their mothers was changed from *Polytoma* to *Dunaliella*. In the general control experiments in which a continuous diet of *Polytoma* was maintained 177 mothers produced 2500 daughters, of which 2+ per cent were male-producing daughters.

The highest percentage of male-producing daughters which was produced by a change of the diet in the experiments in tables 1 to 3 is 83+ but as high a percentage as 87+ was produced in the experiment in table 4.

If twenty-five of the best experiments in tables 1 to 3 are selected and the percentage computed for the male-producing daughters, it is seen that a change of diet from the *Polytoma* to

TABLE 1

Showing that adult female-producing females of various ages, selected at random from a rotifer culture reared on a Polytoma diet through several generations, can be forced to produce a high percentage of male-producing daughters by being transferred from a Polytoma diet to a Dunaliella diet

EXPERIMENT	CONTROL REARED AND CONTINUED ON A POLYTOMA DIET				ADULT ♀♀s REARED ON A POLYTOMA DIET TRANSFERRED TO A DUNALIELLA DIET			
	Mothers ♀♀	Offspring			Mothers ♀♀	Offspring		
		♀♀	♂♀	1/2 ♂♀		♀♀	♂♀	1/2 ♂♀
24	5	30	2	6+	5	23	19	45
25	5	28	2	6+	5	27	12	30+
26	5	36	0	0	18	130	45	25+
27	2	20	0	0	2	12	8	40
28	5	17	1	5+	5	29	15	34+
29	5	30	0	0	5	20	7	25+
30	5	44	0	0	8	24	56	70
31	4	30	0	0	8	37	21	36+
32	4	24	0	0	20	100	78	43+
33	5	40	0	0	9	32	32	50
34	5	33	1	2+	12	77	49	38+
35	5	47	5	9+	22	54	211	79+
36	5	58	2	3+	20	42	172	80+
37	4	60	0	0	15	50	122	70+
38	5	65	3	4+	16	84	156	65
39	5	104	2	1+	10	64	83	56+
Total.....	74	576	18	3+	180	805	1086	57+

the *Dunaliella* causes the percentage of male-producing daughters to change from almost zero to 63+.

The average size of a family of daughters from each mother was 16+. The size of the families in tables 1 and 2 were intentionally made small because of the labor entailed had they been made larger. In table 3 the mothers seemed unable to bear the strain of being transferred from the *Polytoma* diet to the *Dunaliella* diet for the second time and usually soon died. It is possible that some of them were producers of small families, as Shull has shown in some of his experiments.

TABLE 2

Showing that young adult female-producing females reared on a Polytoma diet will continue to produce female-producing daughters but can be forced to produce a high percentage of male-producing daughters by being put upon a Dunaliella diet and later can be forced to produce again female-producing daughters by being put back upon the Polytoma diet

EXPERIMENT	CONTROL REARED AND CONTINUED ON A POLYTOMA DIET					YOUNG ADULT ♀'S REARED ON A POLYTOMA DIET TRANSFERRED TO FRESH POLYTOMA DIET					SAME ADULT ♀'S TRANSFERRED TO A DUNALIELLA DIET					SAME ADULT ♀'S TRANSFERRED BACK TO A POLYTOMA DIET				
	Young ♀ mothers					Young ♀ mothers					Mothers					Mothers				
	♀	♀	♂	♂	%	♀	♀	♂	♂	%	♀	♀	♂	♂	%	♀	♀	♂	♂	%
40	5	54	0	1		A	5	64	0	0	A	5	26	18	49+					
41	5	58	0	0		A	5	50	0	0	A	4	18	12	40					
42	5	88	0	0		A	5	44	0	0	A	5	22	38	63+					
						B	5	44	0	0	B	5	36	32	47+					
43	5	48	0	0		A	5	45	0	0	A	5	35	13	27+					
44	5	122	32	20+		A	4	16	0	0	A	4	25	45	64+	A	4	66	4	5+
45	5	106	2	1+		A	5	26	0	0	A	5	41	25	37+	A	5	32	0	0
46	5	84	0	0		A	5	16	0	0	A	5	10	26	72+	A	5	24	0	0
						B	1	2	0	0	B	1	9	7	43+	B	1	16	0	0
47	5	99	9	8+		A	5	30	0	0	A	5	15	55	78+	A	5	14	2	12+
48	5	100	0	0		A	5	36	0	0	A	5	34	30	46+	A	5	16	0	0
						B	5	28	0	0	B	5	35	35	50	B	5	26	0	0
49	5	106	0	0		A	5	18	0	0	A	5	34	14	29+	A	5	42	0	0
						B	1	12	0	0	B	1	10	6	37+	B	1	17	1	5+
50	5	72	0	0		A	5	26	0	0	A	5	28	36	56+	A	5	26	0	0
						B	5	28	0	0	B	5	42	26	38+	B	5	24	0	0
Total	55	937	13	4+			71	485	0	0		70	404	118	49+		46	303	7	2+

The temperature was uniform for all the individuals of each experiment. When the individuals were put upon a *Dunaliella* diet they were kept at 25 to 26°C. and the control individuals were also kept at this same temperature. During the other time both before and after the change in diet all individuals were kept at room temperature of 18 to 20°C.

The results of Maupas are very easily explained in the light that is given by these experiments. Maupas probably used a

TABLE 3

Showing that young adult female-producing females reared on a Polytoma diet can be forced to produce a high percentage of male-producing daughters among the first children of each family by being put upon a Dunaliella diet and later these same adult females can be forced to produce a high percentage of female-producing daughters by being changed back to a Polytoma diet and still later they can be forced to produce again male-producing daughters by being put back upon a Dunaliella diet

EXPERIMENT	CONTROL REARED AND CONTINUED ON A POLYTOMA DIET				YOUNG ADULT ♀♀S REARED ON A POLYTOMA DIET TRANSFERRED TO A DUNALIELLA DIET				SAME ADULT ♀♀S TRANSFERRED BACK TO A POLYTOMA DIET				SAME ADULT ♀♀S TRANSFERRED AGAIN TO A DUNALIELLA DIET			
	Young adult ♀♀s		Offspring		Young adult ♀♀s		Offspring		Adult ♀♀s		Offspring		Adult ♀♀s		Offspring	
	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂
53	5	98	22	A	5	9	39	81+	A	5	28	2	6+			
				B	5	9	47	83+	B	5	17	7	29+			
54	5	106	43+	A	5	21	17	44+	A	5	15	7	31+			
				B	5	11	19	63+	B	5	41	9	18			
55	5	130	00	A	5	17	17	50	A	5	53	7	11+	A	5	75
				B	5	10	18	64+	B	5	51	7	12+	B	5	85
56	5	75	00	A	5	26	22	45+	A	5	36	1	10	A	5	19
				B	5	29	15	34+	B	5	34	2	5+			
57	5	78	11+	A	5	19	37	66+	A	5	14	4	22+	A	1	2
				B	5	27	31	53+	A	5	28	4	12+	B	4	25
58	5	142	21+	A	5	15	39	72+	A	5	35	3	7+			
				B	5	36	50	57+	B	5	61	3	4+			
59	5	100	43+	A	5	36	48	57+	A	5	60	0	0			
				B	5	56	56	50	B	5	64	0	0			
60	5	104	00	A	5	25	41	62+	A	5	47	1	2+			
				B	5	22	16	42+	B	5	64	0	0			
61	5	80	00	A	5	30	30	50	A	5	60	0	0			
				B	5	16	12	42+	B	5	42	0	0			
Total	45	913	131+		90	414	554	57+		90	750	60	7+		20	206

mixed food culture of Protozoa. When this food culture was placed at a low temperature of 12 to 14°C. only certain species of Protozoa were active and consequently could be used as food by the rotifers. However, when the temperature of this same food culture was raised to 26 + °C., other species of Protozoa which had been in a quiescent stage while the culture was at 12 to 14°C., now became active and were used as food by the rotifers instead of the Protozoa that were used at the low temperature. Thus by changing the temperature the diet was so markedly changed that it constituted the necessary stimulus upon the mothers for the production of male-producing daughters.

TABLE 4

Showing that the influence of the diet acts upon the mother and not upon the male-producing daughter that causes the daughter to produce males. In other words, the influence of the change of diet acts solely upon the grandmother and causes her to beget male grandchildren. Young ♀♀ reared on a Polytoma diet were transferred to a Dunaliella diet for 27 to 36 hours. At the end of this period they were transferred to filtered culture water and allowed to produce eggs. These eggs were transferred to Polytoma culture in which they hatched and the young females grew to maturity and reproduced while being fed exclusively upon Polytoma.

♀♀ MOTHERS REARED ON A POLYTOMA DIET	OFFSPRING FROM EGGS WHICH DEVELOPED ON A DUNALIELLA DIET BUT FED POLYTOMA AFTER HATCHING		
	♀♀	♂♂	% ♂♂
Several.....	8	16	66+
Several.....	9	63	87+

TABLE 5

As a control for table 4. Young ♀♀ reared on Polytoma diet were transferred to a Dunaliella diet for 28 hours. At the end of this period they were transferred to filtered culture water and allowed to produce eggs. These eggs were transferred to fresh Dunaliella culture in which they hatched and the young females grew to maturity and reproduced.

♀♀ MOTHERS REARED ON A POLYTOMA DIET	OFFSPRING FROM EGGS WHICH DEVELOPED ON A DUNALIELLA DIET AND FED DUNALIELLA AFTER HATCHING		
	♀♀	♂♂	% ♂♂
Several.....	35	25	41+
Several.....	30	10	25

The author has several records of epidemics of males occurring in his experiments during the last eight years at periods when accidental rises of temperatures from 20° to 26 or 27°C . took place and while the food used was a mixed culture of green and colorless Protozoa. These epidemics of males can be explained in the same manner as the results of Maupas. At room temperature certain species of green Protozoa were more or less quiescent but when the temperature rose suddenly to 26°C . all the individuals of these somewhat quiescent green Protozoa became very active and furnished a new diet for the rotifers. The stimulation by this new diet caused the mothers to produce male-producing daughters. After a few hours the temperature sank back to the normal temperature of 20°C . and the remaining green Protozoa again became quiescent and the rotifers were forced to eat the other Protozoa that were normally active at this temperature and which was their regular diet.

It has been previously observed that rotifers in a newly made general culture of manure medium produce a much higher percentage of male individuals than rotifers in an old culture of manure medium. It is generally known that in a newly made hay infusion—and the same is true in a newly made manure infusion—the protozoan fauna fluctuates greatly. At first individuals of certain species may be very abundant and later individuals of other species became very numerous, while the individuals of the earlier-appearing species in the culture become relatively few. Thus there is a never-ending change in the protozoan fauna in a new culture of water and manure. Certain species flourish and are very abundant for a short period and then they disappear and new forms replace them. When rotifers are in such culture water with its varying protozoan fauna they are, of course, subjected to many changes of diet. Some of these changes of diet probably act as a stimulus upon the female rotifers so as to cause them to produce male-producing daughters which produce males in the following generation.

The sporadic production of males in the numerous experiments of various workers who have used mixed protozoan cultures as

food for the rotifers can thus be simply explained. Under certain conditions some of the Protozoa are active and others are more or less quiescent, although they may be reproducing in this quiescent stage. When the conditions are changed, possible in other ways than temperature, the quiescent Protozoa become very active, thus constituting a new diet for the rotifers which eventually causes males to be produced.

It would be interesting to know the effect of a continuous feeding of a new diet upon the rotifers, but unfortunately it has been impossible to use the green *Dunaliella* continuously as a diet. They are the most active in sunlight but during the night they become more or less quiescent and consequently can not be used as a food by the rotifers in any appreciable numbers.

It is very probable that other forms of Protozoa as well as *Dunaliella* have the same stimulating effect upon female-producing females of *Hydatina senta* in causing them to produce male grandchildren because in mixed colorless protozoan cultures epidemics of males often occur. It may be possible to find and cultivate a colorless flagellate which will be even more effective than the green one, *Dunaliella*, which has already been used and caused the female-producing females to yield as high as 87+ per cent of male-producing daughters.

The sex strains of Punnett may be more or less due to the diet used. Punnett states that they were fed upon *Euglena* most of the time but does not state whether the *Euglena* were in pure cultures. In some of the food cultures made this year an undetermined species of *Euglena* was cultivated in the same kind of bouillon solution as was *Dunaliella*. This was an excellent food for the rotifers but it did not stimulate them to produce males, as the *Dunaliella* always did when the rotifers were suddenly transferred to it from a *Polytoma* diet. In a previous paper it has been shown that a constant and uniform food supply caused one family to produce only female offspring through 289 generations, although males were produced in side experiments when the food was changed. Punnett unwittingly may have used a pure *Euglena* culture as food for some of his

strains and mixed cultures for other strains, or he may have used a mixed food culture whose protozoan fauna was stable or varied according to the light conditions or possibly other conditions in the laboratory.

In order to cause rotifer mothers to produce male-producing daughters by changing the diet from *Polytoma* to *Dunaliella* the latter diet must be very abundant so that the mothers may consume enormous quantities of it. A change to a meagre diet of *Dunaliella* causes no male-producing daughters to be produced. This fact that the *Dunaliella* diet must be very copious in order that male-producing daughters may be produced, rather indicates that a sudden change in metabolism in which the processes are carried on at their maximum rate, may be a necessary accompanying stimulus, coupled with the new diet stimulus that causes male-producing daughters to be produced.

SUMMARY

1. In the parthenogenetic reproduction of *Hydatina senta* the influence of the diet acting upon the grandmother determines the sex of the grandchildren.

2. A continuous diet of the colorless flagellate, *Polytoma*, causes female grandchildren to be produced.

3. A sudden change of the diet from *Polytoma* to an abundant supply of the active green *Dunaliella* causes male grandchildren to be produced.

4. The regulation of the sex ratio in the parthenogenetic reproduction of *Hydatina senta* therefore can be controlled by food conditions.

Middletown, Conn.
August 14, 1914

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